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ORIGINAL MANUSCRIPT

Helicobacter pylori, cyclooxygenase-2 and evolution of gastric lesions: results from an intervention trial in China

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Abstract

To investigate the role of cyclooxygenase (COX)-2/prostaglandin E₂ (PGE₂) in the process of Helicobacter pylori-induced gastric carcinogenesis, a prospective study based on an intervention trial was conducted in Linqu County, China. A total of 1401 subjects with histopathologic diagnosis were investigated at baseline, among those, 919 completed subsequent interventions (anti-H.pylori and/or celecoxib treatment). Expressions of COX-2 and Ki-67 were assessed by immunohistochemistry, and PGE₂ levels were measured by enzyme immunoassay before and after interventions, respectively. We found a grade–response relationship between COX-2 expression level and risk of advanced gastric lesions at baseline. Stratified analysis indicated an additive interaction between COX-2 expression and H.pylori infection on the elevated risk of advanced gastric lesions. The odds ratios (ORs) for both factors combined were 9.31 [95% confidence interval (CI): 4.13–20.95] for chronic atrophic gastritis, 16.26 (95% CI: 7.29–36.24) for intestinal metaplasia and 21.13 (95% CI: 7.87–56.75) for dysplasia, respectively. After interventions, COX-2 expression and Ki-67 labeling index (LI) were decreased in anti-H.pylori group (OR: 1.65, 95% CI: 1.36–1.99 for COX-2; OR: 1.78, 95% CI: 1.49–2.12 for Ki-67) or anti-H.pylori followed by celecoxib group (OR: 1.41, 95% CI: 1.17–1.70 for COX-2; OR: 1.63, 95% CI: 1.37–1.94 for Ki-67). PGE₂ levels were decreased in all treatment groups. Furthermore, the regression of gastric lesions was associated with the decrease of COX-2 expression or Ki-67 LI after interventions. Our findings indicate that H.pylori-induced COX-2/PGE₂ pathways play an important role on the progression of precancerous gastric lesions in a Chinese population.

Introduction

Helicobacter pylori has been classified as a class I carcinogen by International Agency for Research on Cancer for the significant association with incidence of gastric cancer (GC) (1). The H.pylori-infected patients showed the higher frequencies of precancerous gastric lesions (2) and a 2-fold to 8-fold increased risk of developing GC (3–6). Our randomized controlled intervention trial in Linqu County, a high-risk area of GC in China, indicated that H.pylori eradication could significantly reduce the risks of precancerous gastric lesions and GC, suggesting that H.pylori eradication is effective in preventing GC (7–9). In 2014, a working group report by International Agency for Research on Cancer recommended that for countries with a high risk of GC, the greatest benefit would be gained by eradicating H.pylori infection (10).
Although the mechanisms underlying H. pylori-associated gastric carcinogenesis are not fully understood, it is widely accepted that H. pylori infection is a major initiator of inflammation in gastric mucosa, which progresses further through the multiple premalignant stages to GC (11–14). Several lines of evidence indicate that H. pylori-induced cyclooxygenase (COX)-2/ prostaglandin E2 (PGE2) pathways may play an important role in gastric carcinogenesis (15,16). The upregulated COX-2 by H. pylori can induce the synthesis of PGE2, and consequently stimulate cell proliferation, inhibit apoptosis and initiate mutagenesis (17–20), which suggested that inhibition of COX-2 could be a target for GC prevention (21–25).

In 2004, we conducted a 2×2 factorial design, randomized and placebo-controlled intervention trial in Linqu to prevent the progression or enhance the regression of advanced gastric lesions by anti-H. pylori treatment/COX-2 inhibitor (celecoxib) alone or two interventions combined (26). Consequent result of 2-year interventions supported the hypothesis that anti-H. pylori or celecoxib alone could enhance the regression of advanced gastric lesions, delaying the gastric carcinogenesis process. However, no additional beneficial effects were observed for anti-H. pylori followed by celecoxib treatment.

We were interested in these findings and believe that molecular assessment might help us to understand the mechanisms of different treatment approaches. It is well understood that COX-2, a molecular mediator, plays a crucial role in the process of H. pylori-induced inflammation and gastric carcinogenesis. However, during the H. pylori-induced COX-2/PGE2 carcinogenesis sequence, the role of COX-2 and the relationship with H. pylori on the progression of gastric lesions and molecular changes after interventions are still unclear. We have a unique opportunity to test the dynamic levels of COX-2/PGE2 and cell proliferation activity in H. pylori-infected subjects before and after interventions, and assess their changes with interventions and evolution of precancerous gastric lesions based on our randomized intervention trial.

### Methods

#### Study population

In 2002, an initial screening program was launched in 12 villages in Linqu County, involving 2813 (89.0%) subjects out of 3161 eligible residents aged 35–64 years. Each subject received an interview, physical examination, carbon-13 urea breath test and upper endoscopy. A total of 2638 subjects completed upper endoscopy and pathological diagnosis.

In March 2004, 1024 participants with H. pylori positive and baseline histology of severe chronic atrophic gastritis (CAG), intestinal metaplasia (IM), indefinite dysplasia (Ind DYS) or dysplasia (DYS) were invited to participate in an intervention trial. The detailed design and results have been described elsewhere (26). Briefly, the trial participants were randomly assigned to two interventions (anti-H. pylori and/or celecoxib treatment) or placebo in a 2×2 factorial design. The anti-H. pylori treatment included omeprazole (20mg), amoxicillin (1g) and clarithromycin (500mg) twice daily for 7 days. And celecoxib treatment involved celecoxib (200mg, Pfizer, New York, NY) twice daily for 24 months. Another upper endoscopy examination and pathological diagnosis in a double-blind fashion were conducted in 919 participants at the end of the trial in April 2006, using the same procedures as the baseline.

For the current study, 1024 H. pylori-positive trial participants were included. Because subjects with superficial gastritis (SG) and normal tissue were not included in this trial, 88 SG/normal subjects were selected randomly according to the frequency of histological lesion at baseline. In addition, to test the interaction between H. pylori infection and COX-2 expression on the progression of gastric lesions, H. pylori negative subjects were enrolled from the baseline screening, with 42 DYS (including all of the H. pylori-negative subjects with DYS) and 247 subjects from CAG to Ind DYS selected randomly by frequency-matching in pathological diagnosis. Thus, a total of 1401 subjects were finally enrolled in this study, including SG/normal (n = 88), CAG (n = 295), IM (n = 364), Ind DYS (n = 524) and DYS (n = 130). COX-2 and Ki-67 expression were detected in all of the subjects at baseline, and 916 participants who completed the same procedures as the baseline.

To assess the changes of PGE2 levels before and after interventions, a total of 268 subjects were randomly selected from 1401 subjects at baseline to take one additional新鲜 biopsy tissue in upper endoscopy examination, among them, 93 were detected PGE2 levels after interventions. A written informed consent was obtained from each participant, and the study was approved by the Institutional Review Board of Peking University Cancer Hospital.

#### Upper endoscopy examination and histopathology

The detailed procedures of endoscopy have been described elsewhere (26). Briefly, upper endoscopy examinations were conducted by four experienced gastroenterologists using fiber-optic or video endoscopes (Olympus). The gastric mucosa was examined, and five biopsies were obtained from standard sites of the stomach according to the Updated Sydney System (27): two from the body, one from the antrum and two from the antrum. For the detection of PGE2, level, one or two additional biopsies were obtained from the lesser curvature of antrum or antral adjacent and frozen in liquid nitrogen immediately. All specimens were reviewed by a panel of three pathologists according to the Updated Sydney System and Padova International Classification (27,28). Each biopsy was given a diagnosis based on the most severe histology found in the biopsy, and each subject was assigned a global diagnosis based on the most severe diagnosis among any of the five biopsies.

#### Immunohistochemical analysis

Formalin-fixed, paraffin-embedded tissue specimens were used to determine COX-2 or Ki-67 expression by immunohistochemical analysis. As described previously (29), a monoclonal mouse antibody to COX-2 (diluted 1:100; Cayman Chemical Co.) or Ki-67 (diluted 1:100; Zhongshan Golden Bridge Co.) was used as primary antibody and incubated at 4°C overnight. Anti-mouse immunoglobulin G was used as a secondary antibody and incubated for 30 min at 37°C, and antibody-binding sites were visualized by DAB kit (Zhongshan Golden Bridge Co). Negative controls were prepared by omitting the primary antibody.

COX-2 immunoreactivity score was calculated as the product of staining intensity and staining area (Supplementary Figure S1A and C). The staining intensity was graded on a scale of four grades: 0, no staining of cells; 1, weak staining; 2, moderate staining and 3, strong staining. The percentage of staining area was graded semiquantitatively on four grades: 0, none or <5% cells; 1, 5–30% positive cells; 2, 30–60% positive cells and 3, ≥60% positive cells. These two variables formed the actual score: score 0 (no staining or intensity 1 under 5% area); score 1 (intensity 1 over 5% or intensity 2 between 0% and 30% intensity 3 under 5% area); score 2 (intensity 2 over 30% or intensity 3 between 5% and 60% area) and score 3 (intensity 3 over 60% area). COX-2-positive expression was defined as above score 1.

Ki-67 labeling index (LI) was assessed by observing 500 cell nuclei in areas of the section with the highest labeling frequency, and the percentage of Ki-67 labeled nuclei was used for analysis (Supplementary Figure S1A and C).
The scores of COX-2 expression and Ki-67 LI were determined by two pathologists without the information of interventions.

**PGE$_2$ enzyme immunoassay**

PGE$_2$ levels were measured by enzyme immunoassay using EIA kit (Cayman Chemical Co.). Briefly, frozen tissues were thawed, weighed, homogenized at 4°C, and then purified with C-18 SPE cartridge (Waters). After the derivatization, the samples were mixed with acetylcholinesterase-labeled tracer and PGE$_2$ antiserum, incubated at room temperature for 18 h and developed with Ellman’s reagent. PGE$_2$ levels were presented as picograms per milliliter of wet tissue.

**Carbon-13 urea breath test**

Carbon-13 urea breath test was performed as reported previously (30). Briefly, after a baseline breath sample had been taken, fasting patients were given 80 mg $^{13}$C-urea (>99%), and an additional breath sample was taken after 30 min. The concentration of $^{13}$CO$_2$ at 30 min that exceeded the baseline >4 parts per 1000 (>0.4%) was regarded as a positive result.

**Statistical methods**

For the data from baseline population, the differences in sex, smoking, drinking and H. pylori infection status among different histological patterns were examined by chi-square test. To estimate the association between COX-2 expression and risk of precancerous gastric lesions, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression, adjusting for age, gender, smoking, drinking and H. pylori status. To evaluate the interaction between COX-2 expression and H. pylori infection, we used dummy variables to compare both H. pylori and COX-2 negative with H. pylori negative and COX-2 positive, H. pylori positive and COX-2 negative, and both positive in a multivariate model. Rothman’s synergy index was calculated to determine a relative scale of measurement of the interaction. As a further test for the relationship between COX-2 expression and PGE$_2$ levels or cell proliferation activity, we first calculated median and interquartile range of PGE$_2$ levels or Ki-67 LI because these data were non-normal distribution in this population, and then tested the differences between degrees of COX-2 expression by Wilcoxon rank-sum test.

To estimate the effects of interventions, each standard site in stomach was assigned a severity score at baseline (A) and end point (B) according to the pathological diagnosis: 0 for SG/normal; 1 for mild or moderate CAG; 2 for severe CAG; 3 for superficial IM; 4 for deep IM; 5 for Ind DYS; 6 for low-grade DYS; 7 for high-grade DYS and 8 for GC. The evolution of gastric lesions for each stomach site was classified into progression (B > A), no-change (B = A) or regression (B < A) group, respectively. We also determined the change of COX-2 or Ki-67 expression after interventions as decrease, no-change or increase group, when COX-2 expression score or Ki-67 LI in end point was less than, equal to or more than that in baseline for each stomach site. Unconditional logistic regression was used to evaluate the associations between interventions and molecular changes, or the relationships between changes of expression and evolution of gastric lesions, adjusting for age, gender, drinking, smoking or baseline gastric lesions. Two-sided P value of <0.05 was considered to indicate statistical significance. These analyses were carried out with Statistical Analysis System Software (version 6.12; SAS Institute, Cary, NC).

**Results**

*H. pylori infection, COX-2 expression and risk of gastric lesions at baseline*

A total of 1401 subjects (724 males and 677 females) at baseline were enrolled, including 88 with SG/normal, 295 with CAG, 364 with IM, 524 with Ind DYS and 130 with DYS, respectively.
Information about age, gender and *H. pylori* status was available on 1401 subjects. Data on cigarette smoking and alcohol consumption were obtained from 1386 and 1358 subjects, respectively. As shown in Table 1, the frequency distributions of gender, mean age, cigarette smoking, alcohol drinking and *H. pylori* infection were significantly different in each category (*P* < 0.01).

We first evaluated the association between COX-2 expression scores and risk of gastric lesions. Five biopsies were collected from five standard sites of the stomach in each subject. A total of 6527 biopsies were finally examined COX-2 expression because 478 biopsies were lost during the examination. COX-2 expression was graded semiquantitatively from score 0 to 3. Table 2 showed the relationship between the score of COX-2 expression and risk of gastric lesions. Compared with SG/normal at score 1, the ORs were increased from 2.17 (95% CI: 1.88–2.49) for those with CAG to 5.16 (95% CI: 3.01–8.86) for DYS. At score 2, the ORs were further increased from 3.76 (95% CI: 2.86–4.93) for those with CAG to 29.49 (95% CI: 15.86–54.84) for DYS. Furthermore, at score 3, the ORs were markedly elevated from 4.78 (95% CI: 2.87–7.96) for those with CAG to 39.83 (95% CI: 25.03–63.39) for IM and to 86.44 (95% CI: 41.03–182.09) for DYS.

The risk of precancerous gastric lesions was further evaluated with stratification by COX-2 expression and *H. pylori* status (Table 3). A total of 1378 subjects were determined COX-2 expression based on a global diagnosis. Compared with SG/normal, the OR of CAG was 1.52 (95% CI: 0.72–3.17) for subjects with COX-2 positive or 3.75 (95% CI: 1.59–8.87) for those with *H. pylori* positive alone. However, the OR was significantly elevated in the subjects with combined COX-2 and *H. pylori* positive (OR: 9.31; 95% CI: 4.13–20.95). There was a synergistic interaction between COX-2 expression and *H. pylori* infection, and a synergy index was 2.54. Similar trends were observed in subjects with IM, Ind DYS and DYS. Among subjects with combined COX-2 expression and *H. pylori* infection, the ORs were markedly increased for those with IM (OR: 16.26; 95% CI: 7.29–36.24), Ind DYS (OR: 68.91; 95% CI: 28.25–168.08) and DYS (OR: 21.13; 95% CI: 7.87–56.75), and the synergy indexes were 2.00 for IM, 4.33 for Ind DYS and 3.52 for DYS, respectively.

The relationships between COX-2 expression and cell proliferation activity or PGE? levels were also investigated at baseline. A total of 6850 biopsies were examined both COX-2 and Ki-67 expressions. Ki-67 LI was positively associated with an increased score of COX-2 expression, the median (interquartile range) percentages of Ki-67 LI were 7.4 (3.8–13.3) at score 0, 12.4 (6.6–20.0) at score 1, 17.7 (11.1–25.8) at score 2 and 18.2 (10.6–27.5) at score 3, respectively (*P* < 0.001). The association between COX-2 expression and PGE? levels was also evaluated in 268 biopsies, and similar trend but no statistical association was found. The median (interquartile range) PGE? levels were 71.2 (20.0–138.5) at score 0, 76.1 (36.0–146.4) at score 1 and 95.8 (53.9–272.4) at score 3, but decreased to 67.3 (26.1–196.5) pg/ml/mg wet tissue at score 2, respectively (*P* = 0.48).

### Table 1. Baseline characteristics of selected subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SG/normal</th>
<th>CAG</th>
<th>IM</th>
<th>Ind DYS</th>
<th>DYS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (SD)</td>
<td>52.0±6.7</td>
<td>49.1±6.5</td>
<td>49.4±6.3</td>
<td>49.3±6.6</td>
<td>51.1±6.6</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Sex (%), Male</td>
<td>N = 88</td>
<td>N = 295</td>
<td>N = 364</td>
<td>N = 524</td>
<td>N = 130</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Female</td>
<td>19 (21.6)</td>
<td>135 (45.8)</td>
<td>208 (67.1)</td>
<td>265 (50.6)</td>
<td>50 (38.5)</td>
<td></td>
</tr>
<tr>
<td>Helicobacter pylori infection (%)</td>
<td>N = 88</td>
<td>N = 295</td>
<td>N = 364</td>
<td>N = 524</td>
<td>N = 130</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td>N = 88</td>
<td>N = 290</td>
<td>N = 362</td>
<td>N = 517</td>
<td>N = 129</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Alcohol drinking (%)</td>
<td>N = 86</td>
<td>N = 284</td>
<td>N = 356</td>
<td>N = 507</td>
<td>N = 125</td>
<td>0.001b</td>
</tr>
</tbody>
</table>

- a: *t*-test.
- b: *χ²* test.

### Table 2. Correlation between the score of COX-2 expression and risk of precancerous gastric lesions

<table>
<thead>
<tr>
<th>Score of COX-2 expression</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>OR (95% CI)</td>
<td>N</td>
</tr>
<tr>
<td>SG/normal</td>
<td>1496</td>
<td>1125</td>
<td>1.0</td>
<td>114</td>
</tr>
<tr>
<td>CAG</td>
<td>503</td>
<td>811</td>
<td>2.17 (1.88–2.50)</td>
<td>153</td>
</tr>
<tr>
<td>IM</td>
<td>165</td>
<td>592</td>
<td>4.64 (3.83–5.62)</td>
<td>197</td>
</tr>
<tr>
<td>Ind DYS</td>
<td>156</td>
<td>535</td>
<td>4.54 (3.71–5.55)</td>
<td>235</td>
</tr>
<tr>
<td>DYS</td>
<td>17</td>
<td>68</td>
<td>5.16 (3.01–8.86)</td>
<td>37</td>
</tr>
</tbody>
</table>

*Unconditional logistic regression, adjusted for age, sex, drinking, smoking and Helicobacter pylori infection.*
data of COX-2 and Ki-67 expression (Figure 1). A total of 4519 and 4478 biopsies were finally examined COX-2 and Ki-67 expression after interventions. As shown in Table 4, compared with placebo group, the proportions of decrease/no change of COX-2 expression were significantly increased in the anti-H. pylori followed by celecoxib group and anti-H. pylori group, and ORs were 1.41 (95% CI: 1.17–1.70) and 1.65 (95% CI: 1.36–1.99), respectively. No statistically significant effects were found for celecoxib treatment group. For Ki-67 expression, a similar trend was observed, and ORs were 1.63 (95% CI: 1.37–1.94) and 1.78 (95% CI: 1.49–2.12) for anti-H. pylori followed by celecoxib and anti-H. pylori, respectively.

For subjects who received anti-H. pylori treatment, the rates of H. pylori eradication were 73.2% (161/220) and 69.5% (162/233) by intention-to-treatment analysis in the anti-H. pylori followed by celecoxib and anti-H. pylori groups, respectively. Compared with unsuccessful eradication group, the proportions of decreased/no-changed expressions of COX-2 and Ki-67 were significantly increased in successful group (OR: 1.37, 95% CI: 1.08–1.74 for COX-2; OR: 1.70, 95% CI: 1.37–2.12 for Ki-67) (Supplementary Table S1).

We further compared PGE$_2$ levels before and after interventions. In 93 subjects with PGE$_2$ levels both before and after interventions, there were 27 subjects in the placebo group, 17 subjects in the anti-H. pylori followed by celecoxib group, 26 subjects in the anti-H. pylori group and 23 subjects in the celecoxib group. The PGE$_2$ levels increased 33.88 in the placebo group, but decreased 15.63, 16.37 and 3.81 pg/ml/mg wet tissue in the other active groups, respectively (Supplementary Table S2). Using the median change of PGE$_2$ in total subjects (−2.86) as a cutoff point, the PGE$_2$ levels were significantly decreased in the anti-H. pylori followed by celecoxib (OR: 47.67; 95% CI: 4.40–516.15), anti-H. pylori (OR: 148.30; 95% CI: 13.80–1594.42) and celecoxib (OR: 50.12; 95% CI: 4.80–523.55) groups.

Relationship between the changes of COX-2/Ki-67 expression and evolution of gastric lesions

To assess the relationship between evolution of gastric lesions and changes of COX-2/Ki-67 levels before and after interventions, the participants were classified into three categories: progression, regression and no change. We found that subjects with decreased or no-changed COX-2 expression had a higher opportunity of regression in all groups. As shown in Table 5, OR was 1.51 (95% CI: 1.14–2.01) for placebo, 1.58 (95% CI: 1.16–2.15) for anti-H. pylori followed by celecoxib, 1.41 (95% CI: 1.17–1.70) for anti-H. pylori, and 1.63 (95% CI: 1.37–1.99) for celecoxib.

Table 4. The association of intervention treatments and changes of COX-2 or Ki-67 expression

<table>
<thead>
<tr>
<th>COX-2</th>
<th>Increase</th>
<th>Decrease/no change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo/placebo</td>
<td>389 (29.2)</td>
<td>758 (23.8)</td>
</tr>
<tr>
<td>Anti-Helicobacter pylori/celecoxib</td>
<td>308 (23.1)</td>
<td>802 (25.2)</td>
</tr>
<tr>
<td>Anti-H. pylori/placebo</td>
<td>272 (20.4)</td>
<td>862 (27.0)</td>
</tr>
<tr>
<td>Celecoxib/placebo</td>
<td>364 (27.3)</td>
<td>764 (24.0)</td>
</tr>
<tr>
<td>Placebo/placebo</td>
<td>615 (28.7)</td>
<td>519 (22.2)</td>
</tr>
<tr>
<td>Anti-H. pylori/celecoxib</td>
<td>465 (21.8)</td>
<td>636 (27.2)</td>
</tr>
<tr>
<td>Anti-H. pylori/placebo</td>
<td>442 (20.7)</td>
<td>683 (29.1)</td>
</tr>
<tr>
<td>Celecoxib/placebo</td>
<td>615 (28.8)</td>
<td>503 (21.5)</td>
</tr>
</tbody>
</table>

*Unconditional logistic regression, adjusted for age, sex, drinking and smoking.
Table 5. The association between changes of COX-2 or Ki-67 and evolution of gastric lesions

<table>
<thead>
<tr>
<th>COX-2 expression</th>
<th>Regression versus no regression</th>
<th>Progression versus no progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N, OR (95% CI)*</td>
<td>N, OR (95% CI)*</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>Decrease/no change</td>
</tr>
<tr>
<td>Placebo/placebo</td>
<td>100/363</td>
<td>1.00</td>
</tr>
<tr>
<td>Anti-H. pylori/celecoxib</td>
<td>78/270</td>
<td>1.00</td>
</tr>
<tr>
<td>Anti-H. pylori/placebo</td>
<td>88/257</td>
<td>1.00</td>
</tr>
<tr>
<td>Celecoxib/placebo</td>
<td>99/330</td>
<td>1.00</td>
</tr>
<tr>
<td>Ki-67 LI</td>
<td>177/567</td>
<td>1.00</td>
</tr>
<tr>
<td>Placebo/placebo</td>
<td>135/409</td>
<td>1.00</td>
</tr>
<tr>
<td>Anti-H. pylori/celecoxib</td>
<td>134/406</td>
<td>1.00</td>
</tr>
<tr>
<td>Celecoxib/placebo</td>
<td>186/561</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Unconditional logistic regression, adjusted for age, sex, drinking and smoking.

for anti-H. pylori followed by celecoxib, 1.41 (95% CI: 1.04–1.90) for anti-H. pylori and 1.74 (95% CI: 1.30–2.31) for celecoxib. Whereas a lower risk of progression was observed in subjects with decreased or no-changed COX-2 expression. ORs were 0.46 (95% CI: 0.35–0.63) for placebo, 0.44 (95% CI: 0.31–0.61) for anti-H. pylori followed by celecoxib, 0.49 (95% CI: 0.35–0.69) for anti-H. pylori and 0.43 (95% CI: 0.31–0.59) for celecoxib. A similar trend was found for Ki-67. For subjects with decreased and no-changed Ki-67 expression, a higher opportunity of regression or a lower risk of progression of gastric lesions was also found (Table 5).

Discussion

In this study, we evaluated H. pylori infection and its interaction with COX-2 expression on the risk of precancerous gastric lesions at baseline, and the relationship between dynamic changes of COX-2 expression on the risk of gastric lesions during the follow-up period. The results of our study were consistent with previous studies that showed a significant reduction in COX-2 expression on the regression of gastric lesions. The decrease of COX-2 expression was associated with a lower risk of progression of gastric lesions.

Helicobacter pylori can induce an inflammatory response in the gastric mucosa and is believed to be a potent stimulator of the innate and adaptive immune responses (31). The host immune response to H. pylori influences the progression of gastric lesions (31,32). In the H. pylori-induced inflammatory responses, COX-2 acts as a key regulator (33–35). However, the role of COX-2 and the relationship with H. pylori on the progression of gastric lesions need to be proven. It is tempting to speculate that H. pylori can induce COX-2/PGE2, which consequently leads to the progression of gastric lesions. Helicobacter pylori eradication and COX-2 inhibition could block or inhibit this process, and the regression of gastric lesions should be associated with the decrease of COX-2/PGE2 levels after interventions.

Several studies including our previous study have indicated that COX-2 expression was present in the stages of IM and DYS, and positively associated with histological status (36–39). However, the interaction with H. pylori infection on the risk of advanced gastric lesions has not yet been explored. On the basis of our study at baseline, we found a grade–response relationship between the level of COX-2 expression and risk of gastric lesions, and ORs for Ind DYS and DYS were 36 and 86 times higher among subjects with strong COX-2 expression than those with mild lesions. Furthermore, a significant interaction between COX-2 expression and H. pylori infection on the elevated risk of gastric lesions was observed, suggesting that COX-2 overexpression plays an important role in the development of H. pylori-induced advanced gastric lesions.

This strong association was further confirmed by our subsequent intervention trial, which is the best way to prove a causal link between H. pylori-induced COX-2 and evolution of gastric lesions. We firstly tested the dynamic changes of COX-2 expression on the same stomach site before and after interventions and found a significant decrease of COX-2 expression in anti-H. pylori or anti-H. pylori followed by celecoxib treatment. Further analysis revealed that the proportion of decreased/no-changed COX-2 expression was significantly increased in successful eradication group than those in unsuccessful group, confirming that H. pylori was an initiator during H. pylori-induced COX-2/PGE2 sequence. However, interestingly, no significant decrease of COX-2 level was observed after celecoxib treatment alone. This result is in line with the mechanism of COX-2 inhibitor, which can inhibit the activity of COX-2 enzyme and subsequent main product PGE2, level (40). For H. pylori-infected subjects, COX-2 expression was upregulated in the gastric mucosa and remained even after celecoxib treatment, whereas with COX-2 activity significantly inhibited. Indeed, we further tested the dynamic changes of PGE2 levels before and after interventions and found a significant decrease of PGE2 level not only in anti-H. pylori or anti-H. pylori followed by celecoxib treatment but also in celecoxib treatment group.

Consequently, we evaluated whether the decrease of COX-2 expression was associated with the regression of gastric lesions. We found that the subjects with decreased or no-changed COX-2 expression had a higher opportunity of regression and a lower risk of progression in all treatment arms or placebo arm. The finding in the placebo arm is of specific interest because it explored the role of COX-2 on the continuous evolution of gastric lesions in a prospective study without any interventions. After 4 years of follow-up (from 2002 to 2006), the subjects with elevated COX-2 expression had a significant increased risk of gastric lesion progression.

It has been reported that COX-2 expression is associated with increased gastric epithelial cell proliferation and inhibited apoptosis during the process of gastric carcinogenesis (17,40).
In our study, we tested the correlation between COX-2 expression and cell proliferation activity and demonstrated that Ki-67 LI was increased remarkably by the level of COX-2 expression. Consequently, Ki-67 LI was decreased significantly by effective interventions and showed same trend of alternation as COX-2. We also found that subjects with decreased or no-changed Ki-67 LI had a higher opportunity of lesion regression, suggesting the effects of COX-2 on the epithelial cell turnover during the early stages of malignant transformation.

An intriguing finding of our intervention trial was that no additional beneficial effect on the regression of gastric lesions was observed for anti-*H. pylori* followed by celecoxib treatment. According to the interaction between *H. pylori* infection and COX-2 expression in association with precancerous lesions at baseline, we anticipated that a combination of treatment with anti-*H. pylori* and COX-2 inhibitor should be more effective to prevent the progression of gastric lesions. However, compared the dynamic changes of COX-2 expression and cell proliferation activity before and after interventions in the different treatment groups, a similar change trend was found in anti-*H. pylori* or anti-*H. pylori* followed by celecoxib group. And PGE2, levels were markedly decreased in anti-*H. pylori* group. Our findings might explain the result from our intervention trial that anti-*H. pylori* showed the most favorable effects on the regression of gastric lesions (26), suggesting that sequential inhibition of COX-2 by celecoxib in subjects with low level of COX-2 expression after anti-*H. pylori* might not obtain further beneficial effect on the regression of gastric lesions. The consistent results from the molecular assessment and gastric histological changes revealed the mechanisms underlying GC prevention by anti-*H. pylori* and COX-2 inhibition.

A few limitations of this study should be addressed. First, since there were very few subjects with normal gastric mucosa in this population (only nine subjects), we combined SG with normal as a reference group. As a result, we could not evaluate COX-2 expression in association with SG. Second, because only nine GC cases were identified in the follow-up study after interventions, we could not assess the association between dynamic changes of COX-2 expression and risk of GC.

In conclusion, we found COX-2 expression and its interaction with *H. pylori* infection were strongly associated with an increased risk of advanced gastric lesions in a grade-dependent manner. Moreover, we demonstrated that COX-2 expressions, PGE2 levels and cell proliferation activities were significantly decreased after interventions in the subjects with histological regression of gastric lesions. Together these results suggested that *H. pylori*-induced COX-2/PGE2 pathways may play important roles in gastric carcinogenesis, and COX-2 expression might serve as an intermediary biomarker in GC prevention.

**Supplementary material**

**Supplementary Tables S1 and S2** and **Supplementary Figure S1** can be found at [http://carcin.oxfordjournals.org/](http://carcin.oxfordjournals.org/)

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