Preliminary Study on the Microbiology of Campylobacter pyloridis and Gastric Histopathology

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Abstract. Biopsy samples were taken endoscopically from the antral-mucosa of 693 patients with peptic ulcer and chronic gastritis presenting dyspepsia symptoms. Campylobacter pyloridis cultures were positive in 59 of 98 (60.2%) cases and histopathologically the organisms were found in 411 of 693 cases (59.3%). Pathologically, Campylobacter pyloridis was positive in 273 out of 300 patients with chronic superficial gastritis (91.0%), in 102 of 249 patients with chronic atrophic gastritis (40.9%), in 36 out of 144 patients with chronic atrophic gastritis with intestinalization or dysplasia (25.0%). We found that there was a significant association between the presence of Campylobacter pyloridis and chronic superficial gastritis, also the degree of lymphocyte infiltration showed a strong inverse association with the presence of Campylobacter pyloridis, suggesting that a local immune response might exert an important action in the eradication of this organism. These findings support the view that Campylobacter pyloridis, may be etiologically related to chronic gastritis and peptic ulceration, even though its role still remains to be determined. (Keio J Med 39 (2): 112-116, June 1990)

Key words: biopsy specimen, campylobacter pyloridis, chronic gastritis, peptic ulcer

Introduction

Spiral Gram-negative bacteria attached to human gastric mucosa were observed by Warren1 and Marshall2 in 1983 in Australia. This organism is now officially called Campylobacter pyloridis*. It belongs to a new genus that differs from, but is closely related to, the Campylobacter genus with regard to both morphology and growth requirements. Interest in the relationships between the bacterial infection and peptic ulcer and gastritis has recently been greatly enhanced by the frequent findings of Campylobacter pyloridis in the surface of the inflamed mucosa in patients with gastritis and peptic ulcer. However, the gastric histopathological association of Campylobacter pyloridis infection and the gastric mucosa has not been studied systematically. There have been an increasing number of papers concerning this organism. Campylobacter pyloridis infection of the stomach mucosa has been associated with gastric and duodenal ulcer, non-ulcerative dyspepsia, and gastritis.3-5 This bacteria has been observed in the nearly normal gastric mucosa in only 0-21% of dyspepsia patients, 56-90% of patients with gastritis and 34-77% of patients with gastric ulcer.6 It can be demonstrated in both the antral and body mucosa and its presence is thought by some authors to correlate with the activity of gastritis, although not all accept this postulation.

From 1986 to 1987, 693 patients with various gastric diseases were investigated to confirm Marshall's report that Campylobacter pyloridis can cause gastritis and peptic ulcers. The work reported here was to study further the relationship between Campylobacter pyloridis micro-biologically and various gastric lesions, especially the effect of De Nol on this organism in patients with peptic ulcer.

Materials and Methods

Patients

693 consecutive patients with epigastric discomforts who were referred for gastroscopy at Xijing Hospital, Xi'an Traditional Chinese Medicine Hospital, Hospital

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* Editorial note: The organism is now called Helicobacter pylori
of Xi’an Jiaotong University and Huasan Factory Hospital of Eastern Suburb of Xi’an were investigated. The total patients included 478 males and 215 females. The age range was from 24 to 68 years old with mean age of 42 years. The patients were divided into the following groups: chronic superficial gastritis (CSG) and chronic atrophic gastritis (CAG) with or without peptic ulcer diagnosed endoscopically and pathologically. The number of patients in the former group was 300 cases, in the latter 393 cases. 144 out of 393 patients with atrophic gastritis were accompanied with gastric gland intestinalization and/or dysplasia histopathologically.

Microbiology; Campylobacter pyloridis culture

Biopsy materials from the gastric mucosa were obtained from the area of the lesser curvature three to five centimetres away from pylorus. We chose chocolate and blood agar as the medium for bacterial culture. An antral mucosal biopsy was placed in transport medium (50% glucose solution mixed with normal saline in a ratio of 1:3) for no more than two hours before microaerophilic culture in the laboratory. Before culture the biopsy specimens were rinsed in normal saline for two to three minutes in order to get rid of other substances adhered to the gastric mucosa specimens. The specimens were then cut thoroughly with an autoclaved small knife. The tissues were cultured on the chocolate agar and incubated at 37°C in a microaerophilic environment for five to seven days. In our experiment the Campylobacter pyloridis was identified as urease positive, catalase positive, Gram-negative curved bacilli.

Light microscopy

Biopsied tissues were fixed in 10% neutral buffered formalin. Specimens were embedded in paraffin with standard procedures, then sectioned and stained with hematoxylin and eosin (HE). As reported previously, hematoxylin and eosin stain, in addition to the Warthin-Starry stain, can be used as a routine method for determining the presence of Campylobacter pyloridis. The sections were examined and graded separately for the presence of lymphocyte (chronic gastritis) and polymorphonuclear leukocyte infiltration (active stage of gastritis). The grades (degree) are as follows: first degree signifies only occasional lymphocytes present in a patchy distribution, second degree signifies lymphocyte infiltration intermediate between grade one and three, and third degree signifies a very dense infiltration of lymphocytes or the formation of lymphoid follicles.

Clinical observations

18 patients with peptic ulcer (10 cases of gastric ulcer and 8 cases of duodenal ulcer) were randomly allocated for treatment with tripotassium dicitrate bismuth (TDB or De-Nol) tablets. Each tablet contains 120 mg of TDB (kindly supplied by Cist-Brocades from Holland). One tablet was taken internally 30 minutes before each meal and one more at bed time for 28 days. A comparative study with gastroscopy and biopsy was carried out before and after the treatment. These results were combined with the observations of Campylobacter pyloridis and degree of inflammatory cell infiltration histopathologically.

All results were compared statistically using chi-square or Student’s t test to find any statistical significance.

Results

Isolation of Campylobacter pyloridis from antral mucosa

<table>
<thead>
<tr>
<th>Pathologic Diagnosis</th>
<th>Number of Cases</th>
<th>Campylobacter pyloridis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP positive %</td>
<td></td>
</tr>
<tr>
<td>Simple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSG</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Gastritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAG</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>CAG with INTES or DYS</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Ulcer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSG</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>CAG</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>CAG with INTES or DYS</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

* P<0.05, compared with other groups. CP=Campylobacter pyloridis; CAG=chronic atrophic gastritis; INTES=intestinalization; DYS=dysplasia; CSG=chronic superficial gastritis.
*Campylobacter pyloridis* was isolated separately from the cultured antral mucosa biopsies in 59 out of 98 patients for a positive rate of 60.2% (Table 1). In 51 patients with simple gastritis, *Campylobacter pyloridis* culture was positive in 32 cases (62.7%). The positive rate was highest in chronic superficial gastritis (91.3%, 21/23). In 47 patients with peptic ulcer, *Campylobacter pyloridis* cultures were positive in 27 cases for a positive rate of 57.4%. In patients with peptic ulcer complicated with superficial gastritis, the positive rate was only 50% (14/28). The organisms were usually found to form visible colonies within three to five days after incubation. In our experiment, the bacteria were Gram negative, urease positive, oxidase positive, catalase positive, H$_2$S positive and did not ferment glucose.

**Campylobacter pyloridis in sections of gastric biopsies**

It was revealed that *Campylobacter pyloridis* was always found in sections of antral mucosa biopsies into patients with chronic gastritis, especially in patients with chronic superficial gastritis (91.0%, P<0.01), see Table 2.

<table>
<thead>
<tr>
<th>Pathologic Diagnosis</th>
<th>Number of Cases</th>
<th>Campylobacter pyloridis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSG</td>
<td>300</td>
<td>273</td>
</tr>
<tr>
<td>CAG</td>
<td>249</td>
<td>102</td>
</tr>
<tr>
<td>CAG with INTES or DYS</td>
<td>144</td>
<td>36</td>
</tr>
</tbody>
</table>

\[
P < 0.01
\]

Table 2 Campylobacter pyloridis in sections of antral mucosal biopsies

The relationship between *Campylobacter pyloridis* infection and lymphocytic infiltration

We studied the relationship between *Campylobacter pyloridis* infection and the degree of lymphocytic infiltration statistically (Table 3). The higher the rate of *Campylobacter pyloridis* infection, the less the lymphocytic infiltration.

<table>
<thead>
<tr>
<th>Degree of Lymphocytic Infiltration</th>
<th>Number of Cases</th>
<th>Campylobacter pyloridis</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>114</td>
<td>105</td>
</tr>
<tr>
<td>Second</td>
<td>126</td>
<td>66</td>
</tr>
<tr>
<td>Third</td>
<td>140</td>
<td>30</td>
</tr>
</tbody>
</table>

\[
P < 0.001
\]

Table 3 The relationship between the presence of *Campylobacter pyloridis* and lymphocytic infiltration

The relationship between *Campylobacter pyloridis* infection and lymphocytic infiltration

We studied the relationship between *Campylobacter pyloridis* infection and the degree of lymphocytic infiltration statistically (Table 3). The higher the rate of *Campylobacter pyloridis* infection, the less the lymphocytic infiltration.

**TDB therapy and Campylobacter pyloridis infection in peptic ulcer.**

18 patients were included in this trial. Before the treatment with TDB, 15 cases were *Campylobacter pyloridis* positive. After TDB treatment for 28 days, complete ulcer healing was encountered in 17 cases, for a healing rate of 94.4%. The remaining patient had combined duodenal and gastric ulcers. He presented with duodenal ulcer healing and a decrease in gastric ulcer after four weeks of treatment with De Nol. *Campylobacter pyloridis* was eradicated in 12 out of 15 cases (77.8%), with complete ulcer healing and an improvement in gastritis histopathologically as well (Fig. 2 and 3).

**Discussion**

Chronic gastritis is commonly seen in China and may occur in the pylorus, body or both, with mucosal infiltration of inflammatory cells, hyperemia, edema and mucosal damage with or without glandular atrophy, intestinalization or dysplasia histopathologically. In recent five years, some investigators indicated that *Campylobacter pyloridis*, may be a major factor in the development of gastritis and peptic ulcer.\textsuperscript{8-10} The results in our study show that the positive rates in isolation of *Campylobacter pyloridis* from antral mucosa in patients with chronic gastritis and peptic ulcer are similar (Table 1), but simple gastritis or gastritis complicated with peptic ulcer, of all degrees, showed the highest positive rate of isolation of *Campylobacter pyloridis* in chronic superficial gastritis diagnosed pathologically among all groups of patients (p<0.05). In *Campylobacter pyloridis* positive sections, marked polymorphonuclear cell infiltration is quite common (92.3%). *Campylobacter pyloridis* is strongly associated with chronic superficial gastritis, especially in the acute stage, indicating a close correlation between the *Campylobacter pyloridis* and gastritis. *Campylobacter pyloridis* usually appeared on the surface of the mucosal epithelium, and also in the epithelium (Fig. 1). The results in Table 2 show a statistically significant difference among these defined groups (p<0.01). It is suggested that *Campylobacter pyloridis* is one etiologic factor in the pathogenesis of chronic gastritis. Whether the organism itself can directly cause peptic ulcer must be studied further. There was a decreasing trend in positive rates of *Campylobacter pyloridis* from chronic superficial gastritis (91%) to
chronic atrophic gastritis without (40.9%) and with intestinalization and/or dysplasia (25%) histopathologically. Since the 1900s, it has been suggested that chronic gastritis is a dynamic process that begins as superficial gastritis and progresses slowly to a phase of gastric atrophy. Campylobacter pyloridis might be an initiator in causing gastritis and later other factors, for example an auto-immune mechanism with appearance of PCA, might participate in this process.

There is a very strong inverse association between the presence of Campylobacter pyloridis and the degree of lymphocytic infiltration which has not been reported so far. The greater the lymphocytic infiltration, fewer Campylobacter pyloridis are present. We therefore considered that a local immune responses might play an important role in the eradication of Campylobacter pyloridis.

As reported by other authors, the organism is sensitive to TDB. Although H2 receptor antagonists produce symptomatic and endoscopic resolution of peptic ulceration, relapse is highly frequent with these agents in comparison to bismuth subcitrate. 12–15 18 patients were chosen to undertake treatment with TDB for four weeks. After treatment, Campylobacter pyloridis was cleared in 12 out of 15 patients (77.8%), with ulcer healing and improvement of mucosal inflammation histopathologically (Fig. 2 and 3). But the exact mechanism of the effects of Campylobacter pyloridis on gastritis, peptic ulcer healing, and its relapse needs to be investigated further.

Knowledge of Campylobacter pyloridis-associated gastrointestinal disease is still in its infancy. However, key questions have now been addressed phrased (eg transmission pattern and reservoirs) and recent technological advances will permit the execution of definitive investigations. The precise role of these organisms in the process of gastric distress should be delineated in the not too distant future.

References