Role of *Helicobacter pylori* Infection and Chronic Inflammation in Gastric Cancer in the Cardia

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Background: *Helicobacter pylori*-induced gastritis is an important factor for gastric carcinogenesis. However, it is still controversial whether it is also applicable for cardiac cancer development. Recently, we reported that *H. pylori* is an important factor for the induction of cardiac inflammation. We examined the status of *H. pylori*-induced gastritis in patients with cardiac cancer.

Methods: Seventy-five Japanese patients (58 men; mean age, 64.2 years) with cardiac cancer were studied. Cardiac cancer was defined as that mainly located within 2 cm from the squamo-columnar junction (SCJ). Histological gastritis including the cardiac region was evaluated using the biopsy or surgically resected sections. Cardiac inflammation was evaluated at 1 cm distal from SCJ in lesser curvature. Sera were collected and several markers were evaluated. The status of *H. pylori* infection was evaluated by histology and serum antibodies. Expressions of cytokeratins were examined by immunohistochemical analysis.

Results: Out of 75 patients with cardiac cancer, *H. pylori* was positive in 71 (95%) patients. The cardiac inflammation was examined in 30 patients (26 with *H. pylori* and four without *H. pylori* infection) and we found cardiac inflammation was present in all cases with *H. pylori* infection. Histologically, *H. pylori*-related gastritis was also found in the gastric corpus and antrum. Serological data were consistent with the presence of chronic atrophic gastritis. Intestinal metaplasia was found in 18 cases in the cardiac mucosa, and their cytokeratin 7/20 pattern was judged as a gastric pattern in all cases.

Conclusion: *H. pylori* infection is closely associated with cardiac cancer.

Key words: atrophic gastritis – cardiac cancer – Helicobacter pylori – cytokeratin

INTRODUCTION

Chronic gastritis is mainly induced by *Helicobacter pylori* infection, and provides the basis for gastric carcinogenesis (1,2). Many experimental and epidemiological studies revealed that *H. pylori* plays an important role in gastric carcinogenesis, not only of intestinal type gastric cancer, but also of diffuse type (3,4). Especially in non-cardiac cancer, an extremely close relationship was reported between *H. pylori* infection and gastric carcinogenesis (5,6). Control of *H. pylori* infection is now a standard medical strategy to reduce the prevalence of gastric cancer especially in Japan where gastric cancer is still very common (7).

However, in cardiac cancer, the implication of *H. pylori* infection in its occurrence still remains controversial. A meta-analysis of the previous epidemiological studies revealed a negative association between the prevalence of *H. pylori* infection and of cardiac cancer (5). The prevalence of total gastric cancer is expected to be reduced in the near future by the reduced prevalence of *H. pylori* infection (8,9). However, previous reports have indicated that diseases, such as gastro-esophageal reflux disease or cardiac cancer, may increase in the future (10). It will thus become an important question whether the prevalence of cardiac cancer will increase or not.
The etiology of cardiac inflammation (so-called carditis) which could be linked to cardiac cancer remains uncertain. Recently, we examined the pathogenesis of inflammation in the cardiac area in Japanese patients and demonstrated the significance of *Helicobacter pylori* infection in cardiac inflammation (11). However, it is still unclear whether *H. pylori*-related gastritis is really present in patients with cardiac cancer and also whether *H. pylori* is a important factor for cardiac carcinogenesis or not. In addition, the criterion of the ‘cardiac region’ is still uncertain, which is another important issue in this field.

In the present study, we attempted to clarify the histological and serological background of gastric inflammation in patients with cardiac cancer. Based on these data, we discussed the implication of *H. pylori* infection in cardiac carcinogenesis in Japanese patients.

**PATIENTS AND METHODS**

**SUBJECTS**

Seventy-five Japanese patients (58 men, 17 women; mean age, 64.2 years; range, 25–91 years) were enrolled in this study. In the present study, cardiac cancer was defined as that mainly located within 2 cm from the squamo-columnar junction (SCJ), as described by Siewert et al. (12). Patients treated by previous gasterectomy or the administration of non-steroidal anti-inflammatory drugs or proton pump inhibitors were excluded. Those having undergone successful eradication therapy for *H. pylori* were also excluded. Cardiac cancers were treated by surgical resection or endoscopic mucosal resection (EMR). Informed consent was obtained from all patients, and the ethics committee of Hiroshima University Hospital approved our protocol.

**HISTOLOGICAL EXAMINATION**

Gastritis was evaluated histologically using surgically resected specimens or biopsy specimens. We selected the biopsy or surgically resected sections of the gastric corpus and the antrum in the lesser curvature that were suitable for evaluation of gastritis histologically. The sections that were influenced by the gastric lesions were excluded from our study. The appearance of gastric inflammation was scored using the updated Sydney system (13). For the assessment of cardiac inflammation, we chose the biopsies or surgically resected sections from 1-cm distal to the SCJ on the lesser curvature, and histological gastritis was estimated in the same manner. In the cardiac biopsy, we classified specimens into two groups on the basis of the predominant gastric gland: fundic gland (FG)-dominant or simple mucinous gland-dominant (11). We excluded the section that was influenced by the invasion of tumor tissue in the gastric cardia.

**HELICOBACTER PYLORI INFECTION**

The presence of *H. pylori* was determined by histological examination and assessment of serum antibodies against *H. pylori* (E-plate, Eiken, Japan). In *H. pylori*-negative patients, we confirmed that both tests were negative and that there was an absence of histological gastritis in the gastric corpus and antrum.

**MEASUREMENT OF SERUM PEPsinogen, GASTRIN AND ANTI-PARIETAL CELL ANTIBODY**

Fasting sera were collected from 34 patients upon entry into the study. The sample was centrifuged immediately at 4°C and stored at –20°C until use. Serum concentrations of pepsinogens (PGs) and gastrin were determined by modified radioimmunoassay (14). The serum titer of anti-parietal cell antibody (APCA) was evaluated with the enzyme-linked immunosorbent assay as previously described (15).

**IMMUNOHISTOCHEMICAL ANALYSIS**

Four-micrometer sections of formalin-fixed paraffin-embedded tissues were used for immunohistochemical staining. After deparaffinization and hydration, internal peroxidase was blocked by incubating with 0.3% H2O2 in methanol for 15 min. After incubation with 5% skim milk/PBS, the sections were reacted with the primary antibody (diluted with PBS) for 2 h at room temperature. The primary antibodies used were anti-cytokeratin 7 polyclonal antibody (DAKO, Kyoto, Japan), and anti-cytokeratin 20 polyclonal antibody (DAKO, Kyoto, Japan) (16,17). We performed the immunostaining using an LSAB2 kit (DAKO, Kyoto, Japan). The staining pattern was classified into two patterns (gastric type and Barrett type), as illustrated in the previous report (18).

**STATISTICS**

Results were reported as the mean ± standard error (SE). We assessed the significance of the difference by Mann–Whitney U-test, t-test and χ2-test with StatView software (SAS Institute Inc., Cary, NC). A P value of <0.05 was considered significant.

**RESULTS**

**CLINICOPATHOLOGICAL FINDINGS IN PATIENTS WITH CARDIAC CANCER**

The clinical features of 75 patients with cardiac cancer are summarized in Table 1. Forty-four of the 75 (59%) were diagnosed as early gastric cancer, which limited within submucosal layer. Out of 44, 33 were diagnosed as mucosal cancer preoperatively and treated by endoscopic mucosal resection (EMR). We examined the status of *H. pylori* infection and 71 (95%) were regarded as *H. pylori*-positive. In four patients without *H. pylori* infection, three tumors were diagnosed as the intestinal type and one as a diffuse type cancer.
histological inflammation in sections without \textit{H. pylori} infection (Relation Between \textit{H. pylori} Infection and Cardiac Inflammation)

We then examined the status of histological inflammation in 30 cardiac mucosal sections. In the histological evaluation of cardiac mucosa, we excluded the sections showing the involvement of benign or malignant lesions. The results, including the sub-classification by the \textit{H. pylori} status, are shown in Table 2. In \textit{H. pylori}-positive patients, we detected statistically higher degrees of lymphocyte infiltration in the cardiac mucosa than those in \textit{H. pylori}-negative patients ($P < 0.05$). Neutrophil infiltration, atrophy and intestinal metaplasia in the cardiac region were only detected in \textit{H. pylori} positive patients. Fundic gland (FG)-dominance featured histologically in 35% of cases in \textit{H. pylori} positive group; whereas, all \textit{H. pylori}-negative samples were FG-dominant. When we defined the histological gastritis as that with mild/no atrophy and lymphocyte infiltration without neutrophil infiltration or intestinal metaplasia (19), histological gastritis in the gastric cardia was present in all sections with \textit{H. pylori} infection; whereas, none showed histological inflammation in sections without \textit{H. pylori} infection ($P < 0.01$).

RELATION BETWEEN CARDIAC AND CORPUS/ANTRAL INFLAMMATION

We examined the background gastritis in patients with cardiac cancer both histologically and serologically. As for histological examination, we could collect biopsy samples from the gastric corpus and antrum simultaneously only from 25 patients whose sections from the gastric corpus/antrum in the lesser curvature has a high quality for scoring gastritis. As demonstrated in Table 3, we could detect a higher degree of histological gastritis not only in the corpus, but also in the antrum. The grades of gastritis (atrophy, lymphocyte infiltration, neutrophil infiltration and intestinal metaplasia) were prominent in patients with \textit{H. pylori} infection. Especially, we found the higher grades of intestinal metaplasia in the gastric corpus. As for serological data from 35 patients, the mean values of serum pepsinogen (PG) I, PG II, PG I/II and gastrin were 55.6 ng/ml, 21.9 ng/ml, 2.54 and 211.9 pg/ml, respectively. A low level of pepsinogen I (PG-I) and I/II ratio, and a high level of gastrin were found, and these suggest the presence of corpus atrophic gastritis. The serological status of the APCA level, which is considered to be an inducible factor for corpus atrophy, was not different between the non-neoplastic control groups (data not shown).

### Table 2. Status of histological inflammation of cardiac mucosal sections

<table>
<thead>
<tr>
<th></th>
<th>\textit{H. pylori} (n = 26)</th>
<th>\textit{H. pylori} (n = 4)</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy</td>
<td>1.96 ± 0.13*</td>
<td>0*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocyte infiltration</td>
<td>1.62 ± 0.11*</td>
<td>0.67 ± 0.33*</td>
<td>0.01</td>
</tr>
<tr>
<td>Neutrophil infiltration</td>
<td>0.50 ± 0.14</td>
<td>0</td>
<td>0.23</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>1.19 ± 0.21</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>Fundic gland dominancy</td>
<td>9/26</td>
<td>4/4</td>
<td>0.06</td>
</tr>
<tr>
<td>Cardiac inflammation*</td>
<td>26/26*</td>
<td>0/4*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Defined as described in the text. 
$^*P < 0.01$

### Table 3. Histological gastritis of corpus/antral mucosa in patients with cardiac cancer

<table>
<thead>
<tr>
<th></th>
<th>Antrum (n = 25)</th>
<th>Corpus (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy</td>
<td>2.04 ± 0.21</td>
<td>1.80 ± 0.17</td>
</tr>
<tr>
<td>Lymphocyte infiltration</td>
<td>1.44 ± 0.14</td>
<td>1.44 ± 0.14</td>
</tr>
<tr>
<td>Neutrophil infiltration</td>
<td>0.48 ± 0.10</td>
<td>0.44 ± 0.14</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>2.04 ± 0.25</td>
<td>1.36 ± 0.22</td>
</tr>
</tbody>
</table>

Scores: mean ± SE.

DISCUSSION

Although \textit{H. pylori} is thought to be an important factor for gastric carcinogenesis, it is still controversial whether it...
holds true for cardiac cancer. Previous meta-analysis demonstrated a weak association between \textit{H. pylori} infection and cardiac cancer (5). However, the major problem in this field is the uncertainty of definition of the term ‘cardiac cancer’. The most popular criterion was that designed by Siewert, which defines cardiac cancer as that located within 2 cm of the SCJ (12). Therefore, in the present study we defined ‘cardiac cancer’ as that mainly located within 2 cm of the SCJ.

This area is known as the ‘cardiac area’; however, it is unclear whether true cardiac glands are present in this area. Recent studies have indicated that true cardiac glands are extremely limited, to less than 5 mm from the SCJ (20). We have also reported to find the intact oxyntic gland on the cardiac region (1 cm from ECJ) in sections without inflammatory change and simple mucinous glands could be detected only in cases with chronic inflammation with \textit{H. pylori} infection in Japanese patients (11). Therefore, we suggest that these simple mucinous glands are not true cardiac glands but metaplastic mucosa from oxyntic glands.

It is likely that cardiac cancer comprises two different types; one is the cancer from true cardiac mucosa (with cardiac gland) whereas the other is derived from the gastric corpus (metaplastic) epithelium with chronic inflammation induced by \textit{H. pylori} infection. The proportion of these two may be different between countries or races. In the present study, we collected relatively large numbers of cardiac cancer and examined the background mucosa of these patients. We could demonstrate that most patients with cardiac cancer belonged to the latter group; that is, the cancer derived from metaplastic cardiac mucosa with \textit{H. pylori} infection. Our histological and serological data demonstrated that these patients had pan-gastritis with \textit{H. pylori}-related gastric inflammation from the cardia to corpus/antrum. From our point of view, the carcinogenesis of cardiac cancer may be tightly related to \textit{H. pylori} infection and its related gastritis. Moreover, the major part of gastric cancer derived from this point examined (1 cm from ECJ) should not be considered as true cardiac cancer but proximal cancer derived from the fundic area.

Intestinal metaplasia could be detected in half of all cardiac sections examined, the origin of which is still under debate. It may be derived from the cardiac region or from metaplastic change from the oxyntic gland like a pseudopyloric metaplasia. As described in our results, immunohistochemical analysis revealed that a major part of cardiac mucosas with intestinal metaplasia was of gastric type.

<table>
<thead>
<tr>
<th></th>
<th>Cardiac cancer</th>
<th>Non-cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardia</td>
<td>( n = 18 )</td>
<td>( n = 23 )</td>
</tr>
<tr>
<td>Barrett type</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Gastric type</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Corpus</td>
<td>( n = 8 )</td>
<td>( n = 15 )</td>
</tr>
<tr>
<td>Barrett type</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastric type</td>
<td>8</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4. Expression of cytokeratin 7/20 in gastric mucosa

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Figure 1. Immunohistochemical images for cytokeratin expression; CK-7 (A,C) and CK-20 (B,D). Representative immunostaining pattern of the gastric pattern (A,B) and Barrett pattern (C,D) are demonstrated.
not the Barrett pattern. In addition, these patients showed non-specific gastric inflammation in both the gastric corpus and antrum, compared with dyspepsia patients with H. pylori infection. This suggests that, in the majority of cardiac cancer cases, or at least in our subjects, the mataplastic mucosa is closely associated with H. pylori-related chronic gastritis. Recently, Yagi et al. also reported that the intestinal metaplasia in the cardiac area (5 mm from ECJ) is associated with H. pylori infection and its related gastritis in Japanese patients (21). However, the specificity of the cytokeratins were controversial and some recent studies have raised the question as to its specificity (22).

The problem in our study is the limited examination point of the cardiac region. We only examined the histological gastritis in the lesser curvature, but cardiac cancer could be developed from any region of the cardiac area. We shall examine the difference in the implication of H. pylori in cardiac inflammation between cardiac mucosas from lesser and from greater curvature in the next step. Also, the extension of the true cardiac gland should be defined, especially in H. pylori-negative stomach.

The prevalence of H. pylori infection is decreasing gradually in the Japanese population, which may lead to the reduced prevalence of gastric cancer. However, the true trend of the incidence of cardiac cancer is still uncertain. From our data, we should conclude that cardiac cancer (defined mainly as that located within 2 cm of the ECJ) will decrease along with non-cardiac cancer in the future. In our case examined, only limited cases with cardiac cancer were considered to be H. pylori-independent and these may increase in the future (23). Further studies should focus on the analysis of these H. pylori-independent cardiac cancers, since it will provide useful information for effective cancer screening. Recent advances in magnifying endoscopic examination will provide useful information in this field (24).

Taken together, our present results suggest that a major part of cardiac cancer is mainly induced by H. pylori infection in Japanese patients. The treatment of chronic inflammation may therefore lead to the control of this kind of gastric cancer.

Acknowledgment
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Conflict of interest statement
None declared.

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