Influence of smoking and alcohol on gastric chemokine mRNA expression in patients with Helicobacter pylori infection

T Shimoyama, S M Everett, S Fukuda, A T R Axon, M F Dixon, J E Crabtree

Abstract

Aim—Chemokines that play a primary role in active inflammation are increased in gastric mucosa infected with Helicobacter pylori. Cigarette smoking increases the risk of peptic ulcer disease and gastric cancer, whereas alcohol might exert an antibacterial role. The aim of this study was to examine the association between smoking or alcohol consumption and mucosal chemokine mRNA expression in H pylori associated gastritis.

Methods—Gastric biopsy specimens were obtained from 46 patients with dyspepsia who were infected with H pylori, and total RNA was extracted. Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed to quantify the mRNA expression of three C-X-C chemokines (interleukin 8 (IL-8), growth related oncogene α (GROα), epithelial neutrophil activating protein 78 (ENA-78)) and two C-C chemokines (regulated on activation normal T cell expressed and secreted (RANTES) and monocyte chemotactic protein 1 (MCP-1)).

Results—GROα and ENA-78 mRNA expression was significantly increased (p < 0.05) in 22 smokers compared with 24 non-smokers; however, no difference was seen in the expression of IL-8, RANTES, and MCP-1 mRNA. No differences were observed in chemokine mRNA expression in relation to alcohol consumption.

Conclusions—The increased C-X-C chemokine mRNA expression seen in smokers might play a role in inducing enhanced inflammatory activity in gastritis and the consequent severe diseases associated with H pylori infection.

Keywords: Helicobacter pylori; C-X-C chemokine; smoking; alcohol

The chemokines are a family of inflammatory cytokines with leucocyte chemotactic and activating properties, which play a major role in regulating leucocyte populations migrating into tissues. The chemokine superfamily has been divided into two major subgroups: the C-X-C and the C-C chemokines. C-X-C chemokines, which primarily affect neutrophils, have been detected in higher concentrations in gastric mucosa infected with Helicobacter pylori. Furthermore, semiquantitative analysis of C-X-C chemokine mRNA expression significantly correlates with the severity of gastritis. Infection with cagA positive H pylori induces higher gastric C-X-C chemokine mRNA expression, which might be relevant to the increased mucosal damage associated with cagA positive strains.

The possible effects of lifestyle factors have been studied in upper gastrointestinal diseases. Smoking is thought to affect gastric mucosa adversely by several mechanisms, and is a well established risk factor for peptic ulcer disease and gastric cancer. On the other hand, recent studies have suggested a protective effect of alcohol consumption in H pylori infection. In the lungs, smoking has been linked to raised cellular cytokines, but the effects of lifestyle factors on gastric mucosal chemokine mRNA expression in H pylori infection have not been studied.

The aim of our study was to investigate whether smoking and alcohol consumption influence C-X-C chemokine mRNA expression in H pylori infected gastric mucosa. We studied three C-X-C chemokines: interleukin 8 (IL-8), growth related oncogene α (GROα), epithelial neutrophil activating protein 78 (ENA-78) and two C-C chemokines: regulated on activation normal T cell expressed and secreted (RANTES) and monocyte chemotactic protein 1 (MCP-1) using semiquantitative reverse transcription-polymerase chain reaction (RT-PCR).

Table 1 Patient characteristics in the groups studied

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Sex (M/F)</th>
<th>cagA +/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker</td>
<td>50.8 (4.1)</td>
<td>13/9</td>
<td>14/8</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>47.2 (2.2)</td>
<td>10/14</td>
<td>13/11</td>
</tr>
<tr>
<td>Drinker</td>
<td>45.7 (2.8)</td>
<td>9/5</td>
<td>9/5</td>
</tr>
<tr>
<td>Non-drinker</td>
<td>50.5 (2.9)</td>
<td>14/18</td>
<td>18/14</td>
</tr>
</tbody>
</table>

*Values are expressed as mean (SD).

Materials and methods

Patients with dyspeptic symptoms who were to undergo upper gastrointestinal endoscopy at Leeds General Infirmary were enrolled prospectively into the study. Patients were excluded if they were taking steroids or non-steroidal anti-inflammatory drugs or had received anti-ulcer agents or antibiotics during the two months before endoscopy. Infection with H pylori was confirmed by a rapid biopsy urease test and histology. A total of 83 patients underwent endoscopy. Forty six patients were infected with H pylori and hence eligible for inclusion. There were 23 men and 23 women and the mean age was 49.4 years (table 1).
**Short report**

**Table 2** Ratios of chemokine to glyceraldehyde 3-phosphate dehydrogenase (G3PDH) mRNA expression in the antral mucosa

<table>
<thead>
<tr>
<th></th>
<th>IL-8</th>
<th>GROα</th>
<th>ENA-78</th>
<th>RANTES</th>
<th>MCP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker</td>
<td>1.15</td>
<td>1.45</td>
<td>1.07</td>
<td>0.48</td>
<td>0.74</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>1.00</td>
<td>1.05</td>
<td>0.62</td>
<td>0.44</td>
<td>0.73</td>
</tr>
<tr>
<td>Drinker</td>
<td>1.08</td>
<td>1.23</td>
<td>0.79</td>
<td>0.44</td>
<td>0.67</td>
</tr>
<tr>
<td>Non-drinker</td>
<td>1.07</td>
<td>1.27</td>
<td>0.87</td>
<td>0.47</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SEM).

* Differences are significant between smokers and non-smokers (p < 0.05).

**Discussion**

It is well established that smoking is associated with an increased risk of peptic ulcer disease and gastric cancer. Several studies have investigated the mechanisms by which smoking might induce gastric mucosal injury. The potentiating effects of smoking on gastritis caused by *H pylori* infection are thought to relate to the increased production of oxygen derived free radicals and a decrease in gastric mucus content. Active *H pylori* gastritis is
chemokines can also activate neutrophils to induce epithelial chemokines by expression. VacA has no direct role in the induction of C-X-C chemokine component of smoking, might indirectly play a role in non-smokers. In addition, smoking is associated with increased C-X-C chemokine expression in smokers infected with H pylori-associated gastritis. However, other potential confounding factors, such as dietary antioxidant consumption, should be studied to elucidate the effects of lifestyle on H pylori associated gastritis.

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