Effects of *Helicobacter pylori* eradication therapy on hyperammonaemia in patients with liver cirrhosis

H Miyaji, S Ito, T Azuma, Y Ito, Y Yamazaki, Y Ohtaki, F Sato, M Hirai, M Kuriyama, Y Kohli

**Abstract**

**Background and aims—** *Helicobacter pylori* has strong urease activity. Ammonia produced by *H pylori* in the stomach can be a source of systemic ammonia in patients with hepatic dysfunction. The effect of the eradication of *H pylori* on hyperammonaemia was examined in patients with liver cirrhosis. 

**Methods—** Ammonia concentrations in blood and gastric juice were analysed in 50 patients with liver cirrhosis and hyperammonaemia. All patients were first treated with a low protein diet, kanamycin, lactulose, and branched chain enriched amino acid solution. Hyperammonaemia remained in 18 patients. These 18 patients were divided into three groups according to the status of *H pylori* infection; those with a diffuse distribution of *H pylori* in the stomach (group I), those with a regional distribution (group II), and those without *H pylori* (group III). These patients were given 30 mg lansoprazole, 1000 mg amoxicillin, and 400 mg clarithromycin or 500 mg metronidazole for two weeks to eradicate *H pylori*.

**Results—** In group I ammonia concentrations in blood and gastric juice were significantly reduced after *H pylori* eradication. The blood ammonia concentrations at 12 weeks after the eradication was still significantly lower than that before eradication. In groups II and III the ammonia concentrations in blood and gastric juice were not significantly reduced after eradication therapy.

**Conclusions—** The diffuse distribution of *H pylori* in the stomach contributes partly to hyperammonaemia in patients with liver cirrhosis, and the eradication of *H pylori* is effective in patients with hyperammonaemia with diffuse *H pylori* infection in the stomach.

Keywords: *Helicobacter pylori*, hyperammonaemia, liver cirrhosis, urease.

Chronic hepatic encephalopathy is a disabling complication of cirrhosis and its management is difficult. Patients have generally been treated with restriction of dietary protein intake and given non-absorbable disaccharides and oral antibiotics to reduce the production and absorption of ammonia. Ammonia is of key importance in the pathogenesis of hepatic encephalopathy and hyperammonaemia in patients with cirrhosis is considered to be produced by bacterial urease in the gut flora. *H pylori*, a Gram negative microaerophilic bacterium that infects the gastric mucosa of humans, is probably the most common infection worldwide. *H pylori* infection is widely accepted as the predominant cause of chronic gastritis and is strongly associated with peptic ulcer, gastric cancer, and mucosa associated lymphoid tissue (MALT) lymphoma. *H pylori* is also known to produce copious amounts of ammonia due to its strong urease activity, many times greater than that of urease positive enterobacteria. Therefore, we hypothesised that gastric ammonia produced by *H pylori* can affect the systemic ammonia concentration in patients with liver dysfunction. Previously, we examined the effect of the instillation of *H pylori* in the stomach on the systemic ammonia concentration in rats with cirrhosis induced by carbon tetrachloride. That study showed that the ammonia concentrations in portal and venous blood significantly increased after the instillation of 1 ml 10^7 colony forming units (CFU)/ml *H pylori* in the stomach of cirrhotic rats, and suggested that the ammonia produced by *H pylori* has a role in the pathogenesis of hyperammonaemia when this organism is widely distributed and is present in large numbers in the stomach, particularly in the presence of liver cirrhosis. We also reported the effect of the eradication of *H pylori* on hyperammonaemia in two patients with cirrhosis, and suggested that ammonia production by *H pylori* in the stomach contributes to the systemic ammonia in patients with liver cirrhosis. However, several investigators have questioned whether the effect of the eradication therapy on hyperammonaemia is due to the eradication of *H pylori* or to the non-specific effect of antibiotic therapy on the ammonia producing gut flora. In the present study, we therefore examined the effect of the eradication of *H pylori* on hyperammonaemia in patients with adequate treatment for gut flora.

**Methods**

We studied 50 patients with liver cirrhosis and hyperammonaemia (31 men and 19 women, 43-83 years old, mean age 63; 19 were Childs-Pugh stage A, 18 were B, and 13 were C). All had normal renal function and had never received any anti-*H pylori* treatment. All patients were admitted to Fukui Medical School. Diagnosis of cirrhosis was carried out.
Effects of eradication therapy of Helicobacter pylori on hyperammonaemia in patients with liver cirrhosis

by history, clinical examination, laboratory findings, and liver biopsy when not contraindicated. This study was performed according to the principles of the Declaration of Helsinki, and consent was obtained from each patient after full examination of the nature and protocol of the study.

All patients were given kanamycin (2000 mg/day) for two weeks before H pylori eradication therapy to reduce the effect of the ammonia producing gut flora on hyper-ammonaemia.\(^6\)\(^\text{-}\)\(^9\) During the examination period lactulose was given to all patients and the amount was adjusted individually to induce two to four bowel movements a day.\(^2\)\(^\text{-}\)\(^5\) Protein intake was restricted to about 40 g daily and there were no changes in diet during the examination period. A branched chain enriched amino acid solution (Aminoleban\(^8\), Otsuka, Tokushima, Japan) was also given intravenously to all patients to correct the plasma amino acid imbalance.\(^2\)\(^\text{-}\)\(^9\) The ammonia concentrations in blood were measured early in the morning and after fasting. H pylori infection was diagnosed by phenol red dye spraying endoscopy, culture, histology, and the presence of serum anti-\(H\) pylori IgG. Subjects who had \(H\) pylori identified by more than two tests were defined as \(H\) pylori positive, and those not found to have \(H\) pylori by any tests were defined as negative. During the endoscopy gastric juice was collected for measurement of ammonia concentration.

Phenol red dye spraying endoscopy is a good indicator for detecting the distribution of \(H\) pylori in the stomach.\(^1\)\(^\text{-}\)\(^3\)\(^\text{,}\)\(^8\)\(^\text{-}\)\(^10\) The procedure was as described previously. The basic principle behind this technique is the same as the biopsy urease test. Phenol red solution (0-05%) containing urea (0-5 M) is sprayed onto the gastric mucosa directly through the endoscope instead of in vitro using biopsy specimens. Ammonia is produced by \(H\) pylori, with its strong urease activity, and the colour of the sprayed dye solution changes from yellow to red in a few minutes. The colour change is classified into three types; diffuse, regional, and unstained. We first examined the relation of the distribution pattern of the endoscopy and the ammonia concentration in gastric juice in 68 healthy volunteers (41 men and 27 women, 43–88 years old, mean age 58-3).

Twenty four of the 50 patients were \(H\) pylori positive and 26 were negative. After treatment with a low protein diet, kanamycin, lactulose, and aminoleban\(^8\), hyperammonaemia was still present in 18 cases (12 were \(H\) pylori positive and six were \(H\) pylori negative). These 12 patients were given 30 mg lansoprazole, 1000 mg amoxicillin, and 400 mg clarithromycin or 500 mg metronidazole daily for two weeks for the eradication of \(H\) pylori. Six \(H\) pylori negative cases were also given the eradication therapy as controls. Four weeks later, after eradication therapy ended, endoscopy was performed again to study \(H\) pylori eradication and also to collect gastric juice to measure the ammonia concentration. The blood ammonia concentration was also measured at 12 weeks after the eradication therapy.

Urease activity of \(H\) pylori isolates derived from 24 patients was also measured as described previously.\(^2\)\(^\text{-}\)\(^4\) \(H\) pylori was isolated from biopsy samples in the patients described above using a sterilised endoscope. \(H\) pylori isolates obtained were cultured on TSA-II agar plates (Nippon Beckton Dickinson, Tokyo, Japan) at 37°C under microaerobic conditions (O\(_2\) 5%, CO\(_2\) 15%, N\(_2\) 80%). \(H\) pylori isolates (\(\leq\)\(^10^5\) CFU) were cultured at 37°C in 20 ml brucella broth containing 5% fetal calf serum. Broth cultures were incubated under microaerobic conditions on a gyraatory shaker at 120 rpm for three days. The growth of \(H\) pylori was calculated by measuring the optical density at 560 nm of broth culture medium between each \(H\) pylori isolate and culture medium only. After a three day culture, pellets were obtained by centrifugation and used for the measurement of urease activity. \(H\) pylori pellets were washed with ice cold phosphate buffered saline three times, and then resuspended in 0-5 ml sonication buffer (20 mM sodium phosphate buffer containing 40 g glycine, pH 8-0 (0-5 mM EDTA)). The pellet was sonicated on ice, then centrifuged at 120 000 rpm in a Beckman TLA120.2 rotor for nine minutes. Urease activity in the supernatants obtained was measured as the ammonia production rate from urea. The reaction was carried out for 30 minutes at 37°C using 400 mM urea solution in 100 mM acetate buffer, pH 5-0. The pH of the reaction mixture before and after each reaction was measured to confirm that it remained constant during the reaction. Ammonia produced in the reaction was estimated by means of the indophenol reaction. A standard curve was constructed from an ammonium sulphate solution.

**Statistical analysis**

Values are presented as mean (SD). The significance of the differences in blood and gastric juice ammonia concentrations was determined by Student’s \(t\) test for paired samples and that of the urease activities of \(H\) pylori isolates by Student’s \(t\) test for unpaired samples. Significance was assigned to values of \(p<0\)\(^\text{.}\)\(^0\)\(^\text{-}\)\(^0\)\(^5\).

**Results**

In 32 out of 50 cases (12 \(H\) pylori positive; seven were Childs-Pugh A, three were B, and two were C, and 20 \(H\) pylori negative; 12 were Childs-Pugh A and eight were B) the ammonia concentration in blood was reduced to the normal concentration after the treatment with a low protein diet, kanamycin, lactulose, and aminoleban.\(^8\) However, hyperammonaemia remained in 18 cases. These 18 cases were divided into three groups according to the status of \(H\) pylori infection in the patients with diffuse distribution of \(H\) pylori in the stomach by phenol red dye spraying endoscopy (group I, six cases; two were Childs-Pugh B and four were C), the patients with regional distribution of \(H\) pylori (group II, six cases; two were Childs-Pugh B and four were C), and \(H\) pylori
Clinical characteristics of study groups (mean (SD))

<table>
<thead>
<tr>
<th>Group</th>
<th>Group II</th>
<th>Group III</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>63.2 (7.3)</td>
<td>59.8 (6.6)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>3/3</td>
<td>4/2</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>57.0 (17.1)</td>
<td>64.3 (26.1)</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>31.8 (14.7)</td>
<td>33.2 (14.7)</td>
</tr>
<tr>
<td>Serum bilirubin (mg/dl)</td>
<td>3.2 (0.8)</td>
<td>3.2 (1.2)</td>
</tr>
<tr>
<td>ALB (g/dl)</td>
<td>3.0 (0.4)</td>
<td>2.8 (0.5)</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td>296 (112)</td>
<td>329 (85)</td>
</tr>
<tr>
<td>Cholinesterase (IU/l)</td>
<td>1.6 (0.9)</td>
<td>1.6 (1.0)</td>
</tr>
<tr>
<td>Platelet (x10^9/µl)</td>
<td>6.9 (1.9)</td>
<td>6.9 (2.4)</td>
</tr>
</tbody>
</table>

AST = Aspartate aminotransferase; ALT = Alanine aminotransferase.

In group I, the blood ammonia concentration was 120.0 (40.5) µmol/l before the conventional treatment to reduce the gut flora (period 1), 94.4 (31.5) µmol/l after the conventional treatment (period 2), 57.8 (25.2) µmol/l after the H. pylori eradication treatment (period 3), and 56.5 (22.8) µmol/l at 12 weeks after the eradication treatment (period 4). In group II, the blood ammonia concentrations were 89.3 (13.8), 62.4 (7.8), 56.7 (16.1), and 64.5 (11.9) µmol/l in periods 1, 2, 3, and 4, respectively. In group III, the blood ammonia concentrations were 90.8 (24.2), 63.2 (13.9), 61.5 (14.7), and 70.0 (17.9) µmol/l in periods 1, 2, 3, and 4, respectively.

The ammonia concentration at 12 weeks after the eradication treatment was still significantly lower than that before therapy (p=0.038). In groups II and III, the ammonia concentration after conventional treatment to reduce the gut flora was significantly lower than that before treatment (p=0.002 and p=0.036, respectively), but the ammonia concentration was not significantly reduced after the eradication of H. pylori (Fig 2).

In the healthy volunteers, the ammonia concentration in gastric juice was significantly higher in the diffuse staining pattern (8015 (2182) µmol/l) than in the regional (3927 (2154) µmol/l) or unstained pattern (1874 (1123) µmol/l) (Fig 3). In patients with liver cirrhosis the ammonia concentration in gastric juice was also significantly higher in the patients with diffuse staining (group I) than in the patients with the regional (group II) or unstained (group III) patterns before the eradication treatment. The ammonia concentration in gastric juice was significantly reduced after the eradication of H. pylori in group I (from 8882 (2809) to 1724 (1152) µmol/l, p=0.001). However, there was no significant decrease in the ammonia concentration in gastric juice after the eradication of H. pylori in groups II (3059 (2118) to 1953 (646) µmol/l, p=0.120) and III (1628 (401) to 1505 (398) µmol/l, p=0.608; Fig 4).

Figure 5 shows the urease activity of H. pylori isolates. There was no significant difference in urease activity of H. pylori isolates between groups I (16.7 (3.9) mU/µg protein) and II (15.0 (5.3) mU/µg protein) (Fig 5).

Discussion

H. pylori infection is a common chronic bacterial infection in humans and is considered to be one of the most important factors in the pathogenesis of peptic ulcer disease in the absence of other precipitating factors. H. pylori is found in around 70% of patients with gastric ulcer and 95%–100% of patients with duodenal ulcer. Cure of the infection heals duodenal and gastric ulcers refractory to antisecretory drugs, speeds up ulcer healing, heals ulcers without concomitant gastric acid suppression, and virtually prevents ulcer...
recurrence. On the basis of the intervention trials, the eradication therapy is now strongly recommended for all patients with peptic ulcer infected with _H. pylori_. 34–37 Forty-eight per cent of the patients with liver cirrhosis in this study had _H. pylori_ infection. In patients with liver cirrhosis the control of blood ammonia concentration is important to prevent hepatic encephalopathy. Ammonia produced by _H. pylori_ urease in the stomach can be a source of systemic ammonia in patients with liver cirrhosis. Previously we reported the effect of the eradication of _H. pylori_ on hyperammonaemia in two patients with hepatic encephalopathy. 26 Quero _et al._ also reported a fall in blood ammonia with the eradication of _H. pylori_, but they described the rise of blood ammonia two months after treatment to baseline values in patients after the eradication of _H. pylori_ and suggested that the effect of the eradication of _H. pylori_ on hyperammonaemia is a non-specific effect of antibiotics rather than an effect of the eradication of the organism. 27 Plevris _et al._ found no significant effect of the presence of _H. pylori_ on blood ammonia up to two hours after administration of oral urea. 28 They also suggested that the improvement seen in our initial report may be attributed to a non-specific effect of antibiotics rather than to an effect of the eradication of _H. pylori_. However, in these studies, the patients had not been sufficiently treated for the gut flora before the eradication therapy for _H. pylori_. In the present study all patients were given kanamycin for two weeks before _H. pylori_ eradication therapy to reduce the effect of the gut flora on hyperammonaemia. Blood ammonia concentration was reduced in all cases by the administration of kanamycin, lactulose, aminolesb, and a low protein diet. This effect seems to be strongly associated with the effect on the gut flora. Even after the treatment for the gut flora, the blood ammonia concentration in patients with diffuse distribution of _H. pylori_ in the stomach (group I) was significantly reduced after _H. pylori_ eradication compared with the concentration after the conventional treatment to reduce the gut flora. The ammonia concentration at 12 weeks after the eradication treatment was still significantly lower than that before therapy. The ammonia concentration in gastric juice was also significantly reduced after the eradication of _H. pylori_. In these patients, therefore, the decrease in blood ammonia after the eradication therapy is most likely dependent on the decrease in ammonia production by _H. pylori_ in the stomach. However, no significant effect of the eradication therapy of _H. pylori_ on hyperammonaemia was seen in _H. pylori_-negative patients (group III) and the patients with regional distribution of _H. pylori_ in the stomach (group II).

The phenol red dye spraying endoscopy is a good method for detecting the distribution of _H. pylori_ in the stomach. Initially, we examined the relation of the distribution pattern of the endoscopy and the ammonia concentration in gastric juice in the healthy volunteers, and found that the gastric ammonia concentration in those with a diffuse distribution pattern was significantly higher than that in the subjects with the regional pattern. In this study the gastric ammonia concentration was also significantly higher in the patients with cirrhosis with a diffuse distribution than in those with a regional distribution. In addition, the urease activity of _H. pylori_ isolates did not differ with group. Therefore, the number of the bacteria might be higher in the diffuse cases than in the regional cases. These findings suggest that the contribution of ammonia produced by _H. pylori_ to the systemic concentration depends on the number of bacteria and their distribution in the stomach. The long term control of recurrent hepatic encephalopathy is difficult. We suggest that eradication of _H. pylori_ to reduce ammonia production in the stomach by the organism is effective in patients with hyperammonaemia with diffuse _H. pylori_ infection in the stomach even after conventional therapy with a low protein diet, antibiotics, lactulose, and branched chain enriched amino acid solution.

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Figure 7

Changes in ammonia concentration in gastric juice before and after eradication therapy for H. pylori. Each point and bar represents the mean (SD) of the cases (group I: n=6, group II: n=6, group III: n=6). *p<0.01.

Figure 8

Urease activity (mU/mg protein) before and after eradication. Group I and II. Each point and bar represents the mean (SD) of the isolates (group I: n=6, group II: n=6).