Helicobacter pylori does not release cysteamine into gastric juice

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Abstract

Aim—To determine whether *Helicobacter pylori* releases cysteamine into gastric juice as cysteamine is known to be ulcerogenic.

Methods—Samples of fasting gastric juice were collected from 22 individuals (four women); 10 subjects were *H pylori* negative. The presence of infection was confirmed by examination and culture of gastric biopsies. Cysteamine in gastric juice was measured by reversed phase gradient high performance liquid chromatography with a detection limit of 10 µmol/l.

Results—Cysteamine was not detected in any of the gastric juice samples or in extracts of cultured *H pylori*.

Conclusions—If *H pylori* produces cysteamine then the amounts produced are insignificant and are unlikely to explain the association between *H pylori* infection and the development of duodenal ulcer disease.

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Keywords: *Helicobacter pylori*; cysteamine; duodenal ulcer

Helicobacter pylori, which colonises the gastric mucosa and is thought to be the cause of the development of duodenal ulcer disease, is a metabolically unusual microorganism.

It has been recognised since the organism was first identified that it possesses marked amounts of urease activity.¹ It appears to use this enzyme to scavenge nitrogen from urea that diffuses into the gastric juice from the blood stream.² Nitrogen from urea is assimilated into amino acids through the action of the glutamate dehydrogenase and glutamine synthetase enzymes, both of which are expressed by *H pylori*.³

During studies that followed the incorporation of ¹⁵N labelled nitrogen from urea into amino acids it was noticed that a significant amount of ¹⁵N labelled nitrogen was incorporated into cysteine. This amino acid is structurally related to cysteamine, which is a moiety incorporated into pantotheine through sulphur containing amino acid metabolism.⁴⁻⁶

Cysteamine itself has been used in rats to induce experimental duodenal ulcer disease.^{7,8} The amine is thought to induce ulcers by depleting the concentration of somatostatin in the gastric mucosa thereby impairing the inhibitory control of gastric acid secretion.⁹ The mechanism by which *H pylori* predisposes

to ulcer disease is also via depletion of mucosal somatostatin¹⁰ and disruption of the somatostatin mediated inhibitory control of acid secretion.¹¹⁻¹³

The observation that sulphur containing amino acids were manufactured in response to urease activity suggested the possibility that cysteamine production and release by *H pylori* might be the mechanism by which infection of the gastric mucosa by this microorganism causes duodenal ulcer disease as well as type B gastritis.

Patients and methods

PATIENTS

Samples of fasting gastric juice were collected from 22 individuals (four women) following passage of a 16 French nasogastric tube (Anderson Inc, New York, USA). Of 22 individuals studied 10 (two women) were *H pylori* negative volunteers, seven (one woman) had *H pylori* infection and duodenal ulcer disease, and five (one woman) had *H pylori* infection but not duodenal ulcer disease. The presence of *H pylori* infection was confirmed by the ¹⁴C urea breath test.

SAMPLES

Fasting gastric juice samples were aspirated into sterile universal containers containing 1 M HCl and placed directly into a freezer at -20°C. Samples were transported for analysis frozen with dry ice and kept frozen at -20°C until analysis.

MEASUREMENT OF CYSTEAMINE

Gastric juice specimens were prepared for high performance liquid chromatography (HPLC) following the amino acid chromatography method developed by Turnell and Cooper.¹⁴ Once samples were prepared, they were immediately injected onto a 7.5 cm 3 µm spherisorb ODS column (HPLC Technology, Macclesfield, UK).

Elution of amino acid peaks was by gradient chromatography using a Shimadzu LC10 AD pump with a Shimadzu RF 535 fluorescence detector (Dyson instruments, Tyne and Wear, UK) set at 230 nm for excitation and 420 nm for emission. The gradient system consisted of solution A (water:propionic acid solution: acetonitrile, ratio 36:10:4) and solution B (water:acetonitrile:methanol:DMSO, ratio 21:10:17.5:1.5).¹⁴ The gradient ran from 100% solution A to 100% solution B in five minute intervals over a total of 30 minutes.

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Results

Cysteamine was not found in any of the gastric juice samples or in a lysate of a laboratory culture of *H pylori*. Colonies of the microorganism were extracted into 200 µl of concentrated hydrochloric acid on four separate occasions. The detection limit of cysteamine by this method was 10 µmol/l and the peak eluted between 11.1 and 11.5 minutes. Each sample was run in duplicate with cysteamine (125 µmol/L) added to the second sample to confirm elution of cysteamine at around 11.1 minutes.

Discussion

This study has demonstrated that infection of the gastric mucosa by *H pylori* does not result in the production of significant amounts of cysteamine in the gastric juice, indicating that this is not the mechanism through which *H pylori* infection causes duodenal ulcer disease.⁷⁻¹³

We chose to investigate cysteamine concentrations in fasting gastric juice samples because we considered that any small amounts of cysteamine present would be easily detectable in the relatively concentrated fasting gastric juice. Gastric juice also has a lower protein content than serum, which would reduce interference in the method from protein. Fasting samples were used because protein or sulphur containing amino acids in the diet could also have confounded attempts to measure small quantities of cysteamine.

It seems unlikely that concentrations of cysteamine below the detection limit of the assay would have been sufficient to cause duodenal ulcer disease, or that small amounts of cysteamine produced by *H pylori* were missed. To induce duodenal ulcers in animal studies cysteamine was administered to rats at a dosage of 300 mg/kg. This was sufficient to cause the depletion of immunoreactive somatostatin in the gastric mucosal cells within four hours of administration.^{7,8} An equivalent dose of cysteamine for a 70 kg man would be 21 g, which if distributed equally, produces a theoretical average concentration of 6.5 mmol/l of cysteamine. The exact distribution of cysteamine following subcutaneous injection in rats is not known but as it is a small uncharged molecule it is likely to be widely and easily distributed through body fluids. As millimolar concentrations of cysteamine were required experimentally to produce ulcers, it seems likely that if

cysteamine were produced by *H pylori* the gastric juice concentrations of this compound should be of the same order of magnitude if it were a factor in the development of duodenal ulcer disease. In addition, cysteamine was not detected in supernatants of cultured *H pylori*.

The depletion of gastric mucosal somatostatin is thought to be an important mechanism by which *H pylori* infection leads to duodenal ulcer disease, however, it cannot be attributed to the effects of bacterial cysteamine production. Other causes of the depletion of somatostatin caused by *H pylori* need to be considered. Possibilities include the effect of *H pylori* ammonia production on antral surface pH¹⁵ or effects of cytokines produced by the mucosa in response to the bacterial infection.

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