Inhibition of urease activity but not growth of Helicobacter pylori by acetohydroxamic acid

J Goldie, S J O Veldhuyzen van Zanten, S Jalali, H Richardson, R H Hunt

Abstract
The in vitro effects of acetohydroxamic acid (AHA), a potent urease inhibitor, were studied to determine the effect on the urease activity and growth of 38 strains of Helicobacter pylori. AHA in concentrations of 50-1000 mg/l had a noticeably reversible inhibitory effect on the urease activity of the organism but no effect on growth.

Helicobacter pylori has a very high urease activity which is thought to be related to its pathogenicity, allowing it to colonise and survive in the harsh gastric environment.

There is a need for a more effective treatment against H pylori because currently available treatments are unsatisfactory.

Acetohydroxamic acid (AHA) is a potent inhibitor of the enzyme urease. AHA has been used in the treatment of urinary tract infections, associated with struvite stone formation, in which urea splitting organisms are important. AHA prevents alkalinisation of the urine by inhibiting urease, thus preventing hydrolysis of urea and subsequent production of ammonia.

The high urease activity of H pylori might be inhibited by AHA and we therefore studied this in vitro to determine whether AHA inhibits urease activity and the growth of H pylori.

Methods
Thirty three recent clinical isolates and five reference strains (obtained from LCDC, Ottawa) of H pylori were grown microaerobically at 35°C for five days. Dense suspensions were made in 3 mmol monobasic sodium phosphate buffer (NaH₂PO₄) containing a concentration of AHA to approximate a final concentration of 10⁸ organisms/ml when...
Results of AHA 100 mg/l suspensions of \( H \) \( pylori \) with buffer but without AHA were included in each test series. The pH of the solutions was \( \pm 6 \).

AHA was prepared in an aqueous solution of 10 mg/ml. Dilutions were made in 3 mmol phosphate buffer (pH 6-0) to final concentrations of 1000, 500, 250, 100 and 50 mg/l.

Twenty five microlitres of \( H \) \( pylori \) suspensions in AHA solutions were inoculated into bottles containing 1.5 ml of buffered urea solution of pH \( \pm 6 \) (solution contains 1 ml of Sigma urea standard solution and 49 ml of 3 mmol phosphate buffer). We tested urease activity in organisms that had grown microaerobically for five days rather than three days because we found the urease production to be greatest at five days. Ammonia concentrations were measured after 30 minutes at room temperature using the Kodak Ektachem chemical analyser. Standard crystalline jackbean urease (sigma) was used as standard. The urease activity of the AHA solutions (nmol/residual NH\(_4\)/l) was compared with control suspension without AHA, and background activity of ammonia was subtracted. The average results of three to five experiments per strain are shown (table). The coefficient of variation of this method was 4%. Urease activity of the suspensions was also checked after four and 24 hours using our own rapid urease test.

Subcultures were made after 24 hours from the inoculated AHA suspensions and control suspensions using chocolate agar and buffered peptone medium with 5% horse serum. Both media were at pH 7-0. Each was incubated microaerobically for five days.

Results

The table shows that AHA inhibited the urease activity of \( H \) \( pylori \). The highest level of inhibition occurred with AHA concentrations of 200-1000 mg/l and as there were only minimal differences among strains the average result for each AHA concentration is shown. The inhibition of urease is only temporary because results of the rapid urease test were positive again after 24 hours. AHA did not affect the survival of the organism.

Discussion

The results show that AHA is a potent inhibitor of the urease activity of \( H \) \( pylori \) but that inhibition is reversible and does not affect the growth of the organism. We wanted to study urease activity during optimal growth conditions of the organism, which is at pH \( 5.0-8.0 \) and is adversely affected by lower pH. Because the organism lives in the gastric mucus where the pH is neutral, these studies were performed at a pH of 6-7.

The characteristic high preformed urease activity of \( H \) \( pylori \) is considered important for its pathogenicity. Pathophysiological mechanisms that have been proposed are hydrogen ion back-diffusion and interference with the trichloroacetic acid cycle. The latter results in a decreased production of adenosine triphosphate (ATP) in aerobic cells which may inhibit normal cell function. Furthermore, urease activity allows the organism to use urea as an important nitrogen source.

The urease of \( H \) \( pylori \) differs from other strong urease producers such as \( Proteus \) \( mirabilis \) in showing much higher affinity for urea and higher activity of the enzyme, which may be necessary for the survival of the organism as urea is less available in the gastric mucus than in urine. Although initially AHA was believed to cause irreversible inhibition of urease activity, later studies, which are confirmed by our study, show that inhibition is temporary. Therefore, the inhibitory effect of AHA is highly specific for urease. The optimal pH of the urease activity is about 8.2 and corresponds to optimal growth conditions of \( H \) \( pylori \). (Goldie J, Hollingsworth J, Hunt RH. \( Campylobacter \) \( pylori \) multidisciplinary workshop, Keystone, Colorado, 1987). Hydroxamic acids probably do not have clinically important antimicrobial activity, although one report indicates that AHA is bacteriostatic against several bacteria. In vitro, both synergistic and antagonistic mechanisms have been found when AHA was combined with other antibiotics against Gram negative urease producing organisms. Furthermore, synergism may be pH dependent, which may be relevant for \( H \) \( pylori \), given that the organism must establish itself in the acidic environment of the stomach.

The mechanism by which AHA inhibits urease is unknown, but because growth is not inhibited it does not inactivate the whole organism. Possible mechanisms include inhibition of enzyme activity or enzyme synthesis, or blocking contact of the enzyme with the substrate urea. It has been shown that the cell wall of the organism is no barrier for AHA.

Our results suggest that AHA when used alone will not result in eradication of the organism because growth is not affected. Inhibition of the urease activity, however, could have an adjuvant therapeutic role in the treatment of \( H \) \( pylori \) by making the organism more susceptible to other antimicrobial agents. Side effects may occur with AHA in up to 30% of patients and include bone marrow depression, Coombs' negative haemolytic anaemia, deep vein thrombosis and thrombophlebitis. These occurred when AHA was
Detection of Helicobacter pylori carriers by discriminant analysis of urea and pH levels in gastric juices

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Abstract
An alternative approach to the problems inherent in current methods for detecting *Helicobacter pylori* carriers—that of being generally time-consuming, expensive, and not sufficiently sensitive—was devised by using the urea concentration and pH levels of gastric juices. A linear discriminant analysis of these variables, measured in 54 patients submitted to digestive endoscopy for gastritis, provided a mathematical formula for assigning the subjects (previously classified by other standard methods) to groups of either positive or negative *H pylori* carriers. The results obtained showed a correct classification in 52 out of 54 cases with only one false negative and one false positive case.

Before starting treatment for *Helicobacter pylori* infection, frequently associated with dyspeptic symptoms, an earlier and reliable diagnosis must be made. The urease test performed on histological specimens, considered valid because of its rapidity, low cost, and easy execution, is not very sensitive: only 62% of positive cases are detected in our laboratory compared with other tests such as culture and microscopical examination of biopsy specimens, brushings, and pellets of gastric juices. This report proposes an alternative approach to the problem based on the use of the discriminant analysis of the urea concentrations and pH in gastric juices.

Methods
Fifty four samples of gastric juice, obtained from patients submitted to digestive endoscopy for gastritis (29 men and 25 women, mean (SD) age 48·3 (5·7) years), were analysed by standard cultural and microscopic methods to identify the presence of *H pylori*. The same samples were tested for urea (Beckman: ASTRA 4, Automated Stat/Routine Analyzer with the urea kit from the same manufacturer) and pH concentrations (pH meter).

For the statistical analysis of the data, a discriminant analysis was used (7M module of the BMDP Statistical Package), which allows individual patients to be assigned to different groups, previously classified by another reference system.

Results
Thirty one of 54 biopsy specimens were...
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