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Antibacterial action of the urease inhibitor acetohydroxamic acid on *Helicobacter pylori*

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Abstract

The urease inhibitor acetohydroxamic acid (AHA) was assessed for its bacteriostatic and bactericidal effects on *Helicobacter pylori*. For eight isolates of *H pylori*, the minimum inhibitory concentration (MIC) was either 200 mg/l or 400 mg/l. Interactions between AHA and antimicrobial drugs used to treat *H pylori* were also determined. For most isolates AHA reduced the MIC for colloidal bismuth subcitrate (CBS), tetracycline, metronidazole, and amoxicillin. In a few isolates, however, AHA increased the minimum bactericidal concentration (MBC) for these antimicrobial treatments. In vitro AHA is active against *H pylori* and it interacts with other agents directed against *H pylori*.

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Helicobacter pylori colonises the human stomach, causes antral gastritis, and is frequently associated with peptic ulcer disease. It produces copious amounts of the enzyme urease which protects the organism from acid. This protection is abolished in vitro by acetohydroxamic acid (AHA).[1 2] It is not known if AHA or other urease inhibitors can be used in the treatment of *H pylori* infections.

Opinion on the antimicrobial properties of AHA varies.[1] It has been reported to be bacteriostatic for several organisms,[3] including *H pylori*,[4] and that it acts synergistically with antimicrobial drugs directed against urease producing organisms other than *H pylori*.[5] Others, however, have found that AHA has no effect on *H pylori*.[6 7] We now report our findings on the bacteriostatic and bactericidal effects of AHA on *H pylori*, and on the interactions between AHA and antimicrobial drugs used to treat the infection.

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Methods

H pylori type strain NCTC 11637 and seven clinical isolates were used for all experiments. Minimum inhibitory and bactericidal concentrations (MIC) (MBC) were determined as described previously.[8] Serial doubling dilutions of colloidal bismuth subcitrate (CBS, Gist-Brocades, the Netherlands), tetracycline (Sigma Chemical Co., St Louis, Missouri, USA), metronidazole (Sigma), amoxicillin (Sigma), and AHA (Sigma) were made in 1 ml of brucella broth (Difco) with 10% fetal bovine serum. *H pylori*, grown for three days in a microaerobic atmosphere on Colombia sheep blood agar, was suspended in broth (about 3×10^6 cfu/ml). One millilitre of this bacterial suspension was added to each antimicrobial dilution and to broth without antibiotic. These were incubated for three days in the microaerobic atmosphere. The MIC was defined as the lowest concentration of antimicrobial drug preventing visible growth.

All bottles without visible growth were subcultured on to blood agar plates. The MBC was defined as the lowest concentration of antimicrobial drug that prevented growth on these plates after incubation for three days.

Interactions between AHA and antimicrobial drugs were investigated by performing serial doubling dilutions of each antimicrobial drug in broth containing a subinhibitory concentration of AHA (0·25 × MIC of AHA for each isolate).

Results

AHA was inhibitory for all isolates and bactericidal for seven of eight isolates of *H pylori* tested. The MIC was either 200 mg/l (two isolates) or 400 mg/l (six isolates). The MBC was 400 mg/l (three isolates), 800 mg/l (three isolates), or 1600 mg/l (one isolate).

MICs and MBCs for CBS, tetracycline, metronidazole, and amoxicillin overlapped previously reported ranges.[8]

Subinhibitory concentrations of AHA caused a two- or more-fold reduction in MIC for CBS in all eight isolates (table). For most isolates, AHA also reduced the MIC for tetracycline, metronidazole, and amoxicillin (table). The MIC was not increased by AHA.

For some isolates, AHA caused a reduction in MBC for tetracycline, metronidazole, and amoxicillin. For a few isolates, the MBC was increased for all four antimicrobial drugs (table).

Effect of AHA on MICs and MBCs of antimicrobial drugs

	MIC		*MBC*	
	Decreased	*Increased*	*Decreased*	*Increased*
CBS	8/8	0/8	0/7	2/7
Tetracycline	5/8	0/8	1/6	3/6
Metronidazole	7/8	0/8	3/8	1/8
Amoxicillin	6/7	0/7	1/7	1/7

AHA = 50 or 100 mg/l. The table shows the number of isolates affected over the number tested.

Discussion

AHA is both bacteriostatic and bactericidal for *H pylori* in broth culture, in agreement with the findings of Glupczynski *et al.*[4] The negative findings of other workers may reflect methodological differences.[6 7]

For most isolate/antimicrobial combinations, subinhibitory concentrations of AHA affected the activity of the antimicrobial drug (table). Although a reduction in MIC for each antimicrobial drug was the rule, these were rarely large, with greater than four-fold reductions occurring only three times. Glupczynski *et al* observed such synergy between AHA and tetracycline and metronidazole for some isolates.[4] Like us, they did not observe any antagonistic effect.

In contrast, we found that MBCs were increased by AHA for seven of 28 isolates/antimicrobial combinations (table), although none of these increases was more than four-fold. MBCs were reduced by AHA for only five of the 28 combinations.

The mechanisms of action of AHA, either alone or in conjunction with antimicrobial drugs, in broth culture at neutral pH, are unknown. At concentrations higher than 1000 mg/l, AHA is bacteriostatic to some organisms that do not produce urease.[3] This non-urease mechanism may also work for *H pylori*. *H pylori* urease is, however, inhibited in vitro at lower concentrations[7]; 50% inhibition occurs at 100 mg/l[6]; and synergistic interactions with antimicrobial drugs also occur at this concentration (table). In vivo a single 750 mg dose of AHA inhibits *H pylori* urease in infected patients by 86%.[9]

Animal models of Helicobacter infection may be useful in further investigations into the clinical potential of urease inhibitors. It is already known that urease inhibitors alone do not clear existing Helicobacter infections.[10 11] No animal studies, however, have combined urease inhibitors with antimicrobial drugs.

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