Effect of the Carbonic Anhydrase Inhibitor, Acetazolamide, on *Helicobacter pylori* Infection in vivo: A Pilot Study

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ABSTRACT

Background. Carbonic anhydrase inhibitors have been successfully used to treat peptic ulcers. Although carbonic anhydrase restriction does not inhibit *Helicobacter pylori* in vitro, recent studies suggest that carbonic anhydrase inhibition reduces the ability of *H. pylori* to survive in an acid environment as present in the stomach.

Methods. In a pilot study, we examined the effect of acetazolamide 500 mg as twice a day for 4 days in volunteers with active *H. pylori* infection. Effectiveness was judged by changes in the results of the urea breath test.

Results. Eight *H. pylori* infected volunteers completed the test. No urea breath test reverted to negative and there was a trend for the urea breath test value to increase [e.g. delta over baseline (DOB) mean ± SE increased from 50.9 ± 13 at baseline to 64.9 ± 13 at day 5] during treatment with acetazolamide.

Conclusion. The potential effect of carbonic anhydrase inhibitors on acid secretion may prevent effect on *H. pylori* in vivo and/or the sites of infection at the surface of the stomach may have a pH higher for any postulated acid-dependent effect to have an effect clinically.

Keywords. *Helicobacter pylori*, treatment, acetazolamide, clinical trial, urea breath testing.

Helicobacter pylori causes a luminal mucosal surface infection of the stomach. It is among the most prevalent of the human pathogens worldwide. The infection has proven difficult to treat and usually requires multiple antibiotics taken concomitantly with an acid-suppressing agent [1]. Treatment success has fallen as antibiotic resistance has emerged as a major public health concern [2] and better therapies are needed.

In the presence of physiological urea concentrations, *H. pylori* is able to adapt to a wide range of acidity by buffering the periplasm and cytoplasm through the expression of an acid-activated urea channel, *Ure*I, and a neutral pH optimum intrabacterial urease [3]. One possibility for a new approach would be to disrupt the pH-homeostatic genes that play an important role in acid survival. *H. pylori* contains both alpha and beta carbonic anhydrase with beta-carbonic anhydrase being localized to the cytosol both on the cytosolic side of the inner membrane and on the outer membrane facing the periplasmic space. The alpha-enzyme is attached to the surface of the bacterium [4].

Scott et al. reported increased expression of *H. pylori* alpha-carbonic anhydrase as the pH decreased to 4.5 [5]. Of interest, an alpha-carbonic anhydrase deletion mutant was unable to buffer either the periplasm or the cytoplasm despite the presence of urea and had impaired survival at pH 2.5 in the presence of urea. A similar effect was seen when the carbonic anhydrase inhibitor, acetazolamide, was added to the wild type *H. pylori* prior to the addition of urea. They suggested that periplasmic carbonic anhydrase was likely to be important and plays a complementary role to urease by trapping bicarbonate ion in the periplasm and hypothesized that carbonic anhydrase inhibitors may be effective for the eradication of *H. pylori* in vivo. Synergy between carbonic anhydrase and urease had been suggested in other bacterial systems [6].

While acetazolamide has been shown to be ineffective with regards to killing *H. pylori* in vitro, [4] the studies by Scott et al. suggest that

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it may be effective in the normal acid environment of the stomach [5]. That hypothesis is also consistent with prior data showing good ulcer healing with carbonic anhydrase inhibition as primary therapy for ulcer disease [7]. We tested their hypothesis by giving acetazolamide to *H. pylori* infected volunteers and monitoring the effect with the urea breath test, a convenient measure of *H. pylori* density and enzyme activity which would be reflected in a decrease in urease activity [8–10].

Methods

Healthy volunteers at least 21 years of age with proven active *H. pylori* infection confirmed by a positive baseline 13C-urea breath tests were enrolled. Exclusion criteria include diabetes, suffering from any serious medical conditions, pregnant, lactating, or were known to be allergic to acetazolamide. Precautions were taken to ensure that the subjects were not concomitantly taking medications that could interact or interfere with acetazolamide or potentially expose the subject to adverse reactions (e.g. sulfonamides, aspirin, lithium) or confound observations including proton pump inhibitors, H2-antagonists or any antibiotic within 30 days of testing.

Acetazolamide (500 mg tablets, Storz Pharmaceuticals) was administered to each subject twice daily prior to meals for a total of 1 g daily for four consecutive days. Urea breath tests were performed each morning while volunteer fasting, and prior to morning dose of acetazolamide. First, a baseline breath test was obtained followed by having each volunteer drink premixed urea solution consisting of 2.1 g of citric acid, 150 mg of aspartame (NutraSweet®) and 125 mg of ^{13}C-urea (Meretek, Lafayette, CO) in 100 ml of water. This solution was comparable to commercial urea breath test and the cut-off value for positive versus negative was a delta-over-baseline (DOB) of 2.4. Breath samples were analyzed using a UBiT-IR300 spectrophotometer (Otsuka Electronics, Japan). The outcome variable was a change in $^{13}CO_2$ enrichment from baseline (delta per mil). The primary outcome variable was the proportion of the subjects who had negative breath test results. The secondary objective was the proportion with a consistent and sustained fall in urea breath values below baseline values.

The protocol was approved by the local institutional review committees and each subject signed informed consent prior to participation. Statistical analysis was by ANOVA for repeated measures.

Statistical analysis

The protocol was approved by the local institutional review committees and each subject signed informed consent prior to participation. The design was to do an interim analysis after approximately 8–10 subjects to test whether there was no effect. Statistical analysis was by ANOVA for repeated measures.

Results

Nine volunteers were enrolled and received the drug; eight completed the study (three men and five women, ages ranged from 33 to 58 years); one withdrew on the second day because of mild perioral paresthesias. No subject met the primary or secondary endpoints (Fig. 1) and in no instance did the urea breath test result revert from positive to negative. There was a trend for the urea breath test value to increase (e.g. DOB: mean ± SE increased from 50.9 ± 13 at baseline to 64.9 ± 13 at day 5) during treatment with acetazolamide ($p = 0.538$).

Discussion

Recent in vitro studies showed that carbonic anhydrase inhibition would increase the susceptibility of *H. pylori* to acid environments and suggested that use of carbonic anhydrase inhibitors

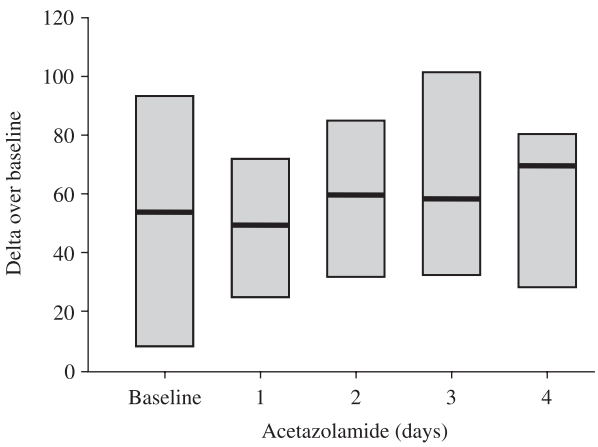

Figure 1 Box plot showing the urea breath test results after 1–4 days of receiving acetazolamide 500 mg twice daily. The ends of the boxes define the 25th and 75th percentiles, with the bold line at the median.

might provide successful monotherapy for *H. pylori* infections [5]. This hypothesis was also consistent with earlier data from large clinical trials showing that carbonic anhydrase inhibitors enhanced the healing of peptic ulcers [7]. We used a clinically high dose of acetazolamide to test the hypothesis that carbonic anhydrase inhibition would inhibit *H. pylori* survival in vivo. We found no evidence for a detrimental effect and paradoxically, found a trend toward higher rates of urea hydrolysis consistent with no effect or a slight increase in *H. pylori* density.

Our results clearly show that acetazolamide had no inhibitory effect on *H. pylori* growth in vivo at the normal intragastric pH. Thus, while carbonic anhydrase inhibitors have been proven effective in the treatment of gastric and duodenal ulcers, the mechanism of action may be limited to an effect on acid secretion and if so, it is possible that the potential effect of carbonic anhydrase inhibitors on acid secretion might prevent them from having an effect on *H. pylori* in vivo. One could also speculate that the site of infection at the surface of the stomach and deep within the gastric pits may provide a site where the pH level is not sufficiently low to allow any postulated acid-dependent effect to become clinically important.

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