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N-Acetylcysteine, a Novel Treatment for *Helicobacter pylori* Infection

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N-Acetylcysteine (NAC), being both a mucolytic agent and a thiol-containing antioxidant, may affect the establishment and maintenance of *H. pylori* infection within the gastric mucus layer and mucosa. Agar and broth dilution susceptibility tests determined the MIC of *H. pylori* strain SSI to NAC. *H. pylori* load in SSI strain-infected C57BL mice was determined as colony forming units per gram of gastric tissue. Gastritis assessment was scored and gastric surface hydrophobicity was determined by contact angle measurement. MICs of NAC were 5 to 10 and 10 to 15 mg/ml using the agar dilution and broth dilution methods, respectively. NAC (120 mg per day for 14 days) reduced the *H. pylori* load in mice by almost 1 log compared with sham treatment. Pretreatment with NAC (40 mg/day) also significantly reduced the *H. pylori* load but did not prevent *H. pylori* colonization. Both *H. pylori* infection and NAC reduced the surface hydrophobicity of murine gastric mucosa. No significant differences were observed in the gastritis scores of *H. felis*- or *H. pylori*-infected mice receiving either NAC or sham treatments. This study demonstrates that NAC inhibits the growth of *H. pylori* in both agar and broth susceptibility tests and in *H. pylori*-infected mice. NAC did not alter the severity of *H. pylori*- or *H. felis*-induced gastritis.

KEY WORDS: *Helicobacter pylori*; mucus; *N*-acetylcysteine; hydrophobicity; treatment.

Helicobacter pylori is one of the most common chronic microbial infection in humans. The prevalence of *H. pylori* varies widely between geographically and ethnoculturally diverse populations. The lifetime risk of infection is approximately 50% in most Western populations and more than 90% in the third world (1). It is responsible for >90% of all duodenal ulcers and 70–80% of gastric ulcers (2). It is a class I carcinogen and a major predisposing factor to the development of gastric adenocarcinoma and MALT lymphoma (3).

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Therapy for *H. pylori* consists of combination therapy comprising either a proton pump inhibitor or bismuth subsalicylate or subcitrate, amoxycillin, or clarithromycin and metronidazole given over a 1- to 2-week period (4). Clearance rates are approximately 80–90% in compliant patients. However, compliance is a major problem because bismuth and metronidazole are unpalatable and a large number of pills must be administered. Also, metronidazole and clarithromycin resistance is emerging, which renders these regimes less effective (4). Prevalence of pretreatment resistance is approaching 15% for clarithromycin and 50% for metronidazole. A recent Australian study has shown that 36% of isolates from patients are now resistant to metronidazole, and 11% to clarithromycin (5). Therefore, novel therapies that are safe, effective, and free of side effects are urgently needed.

The gastrointestinal tract is colonized in abundance by diverse microrganisms, yet *H. pylori* is the only bacterium that commonly thrives in the harsh gastric milieu. *H. pylori*

possesses unique properties for adaptation to this gastric environment. Penetration of mucus to escape the low gastric pH is one of the most important of these factors (6). *H. pylori* induced-gastritis is characterized by marked neutrophilic infiltrate of the gastric mucosa and the generation of reactive oxygen metabolites. Studies in both humans and animals suggest that antioxidants play a role in reducing both gastric inflammation and *H. pylori* load (7). High-intake food-related antioxidants such as vitamin C, α-tocopherol, and β-carotene reduced the prevalence of *H. pylori* infection in Colombian children (8).

N-Acetylcysteine (NAC) is both a mucolytic agent and a thiol-containing antioxidant. NAC possesses a sulfhydryl group which disrupts the disulfide bonds of glycoproteins in mucus and provides a cysteine source for the intracellular synthesis of glutathione (9). NAC, unlike some other bronchial mucolytics such as carbocysteine and bromhexine, has been shown in in vitro studies to change the viscoelastic properties of gastric mucin (10). It also has antibacterial properties (11). NAC has been used in the treatment of cystic fibrosis, lung disease, chronic bronchitis, and paracetamol overdose and in patients with fulminant hepatic failure (12–14.) Accordingly the primary aim of this study was to evaluate the effect of NAC on the growth of *H. pylori* both in vitro and in vivo using mice infected with *H. pylori*. Our secondary aims were to determine the effect of NAC's mucolytic properties on gastric mucosa colonized with *H. pylori* and its antioxidant effect on *Helicobacter*-induced gastritis.

MATERIALS AND METHODS

Murine-adapted *H. pylori* strain SSI and *Helicobacter felis* were employed in this study (15, 16). *H. pylori* were cultured on Columbia blood agar plates containing 10% sheep blood and Dent's antibiotic supplement (Oxoid Australia Pty. Ltd., Victoria, Australia) and then incubated at 37°C under microaerophilic conditions (5% oxygen, 85% nitrogen, and 10% carbon dioxide) for 48 hr. Bacteria were then inoculated into brain heart infusion (BHI; Oxoid Australia Pty. Ltd.) broth supplemented with 5% horse serum and grown overnight with shaking under microaerophilic conditions at 37°C. Bacteria were harvested and resuspended in antibiotic-free BHI. The identity of *H. pylori* from the resuspension was confirmed by testing for urease, catalase, and oxidase activities (17). *H. pylori* morphology and motility were observed under wet preparation microscopy to assess viability. *H. felis* was grown on Columbian blood agar plates containing 10% sheep blood and Skirrow antibiotic supplement (Oxoid Pty. Ltd.). *H. pylori* count was determined using the absorbance from a predetermined standard curve. *H. felis* count was determined by counting bacteria on a hematocytometer.

Agar Dilution Susceptibility Test

Columbian blood agar plates containing 10% sheep blood were supplemented with NAC at concentrations ranging from 0.0625 to 20 mg/ml (Parvolex; gift from FA Faudings Inc., SA, Australia). These agar plates were inoculated with 20 μl of 5×10^8 SS1 bacteria and incubated in a microaerophilic environment at 37°C. Growth of *H. pylori* strain SS1 was assessed on day 5 of incubation and the minimal inhibitory concentration (MIC) of NAC determined.

Broth Dilution Susceptibility Test

Broth dilution susceptibility tests were performed using NAC dissolved in BHI broth at concentrations of 5 to 15 mg/ml. Broth was inoculated with 5×10^8 SS1 bacteria and incubated in a microaerophilic environment at 37°C for 96 hr At 0, 24, 48, 72, and 96 hr, 1 ml aliquots of broth were removed, serially diluted, transferred to Columbian blood agar, and incubated in a microaerophilic environment at 37°C for 5 days. Colony forming units (CFU) per milliliter of broth was determined at each time point.

Murine NAC Trial

C57BL/6 female mice were provided by Animal Facilities, University of Adelaide. Animal experimentation protocols were approved by the Animal Ethics Committee at the Women's and Children's Hospital and the University of Adelaide. Mice were infected with either *H. pylori* strain SS1 (15) or *H. felis* (16).

Protocol for Prevention Trial. Twenty control mice were gavaged with sterile water. Fifteen mice were gavaged twice with 40 mg of NAC per day for a total of 10 days. Mice were gavaged with 5×10^8 *H. pylori* strain SS1 on day 3 of treatment. Mice were sacrificed on day 11.

Protocol for Treatment Trial. Forty mice were gavaged with 5×10^8 *H. pylori* strain SS1 per mouse and infected for 4 months. Twenty mice were gavaged three times a day with 120 mg of NAC per day for 14 days. The 20 controls received sterile water. Mice were sacrificed on day 15.

Protocol for *H. felis* Trial. *H. felis*-infected C57BL/6 mice have more severe gastritis compare to *H. pylori*-infected C57BL/6 mice (18). Thus, *H. felis* was used to assess the effect of NAC treatment on gastritis. Twenty-two mice were infected with 5×10^8 *H. felis* for 12 weeks. Half were treated with NAC for 14 days and the other half received sterile water. Mice were sacrificed on day 15.

Tissue Collection. Mice were anesthetized with halothane and killed by cervical dislocation. The stomach was removed via an abdominal incision; the fundus of the stomach lined with squamous epithelium was removed by cutting along the squamocolumnar junction. The remaining stomach was cut along the greater curvature. Gastric content was removed by washing in sterile normal saline. Using sterile techniques, the stomach was cut into two longitudinal halves for histological assessment and culture.

Culture of *H. pylori* from Gastric Tissue. Half the stomach was weighed and homogenized in normal saline. Serial dilutions of homogenate were transferred to *Helicobacter* selective agar plates (Columbian blood agar with Oxoid Dent supplement SR 147E) and incubated in a 10% CO_2 environment at 37°C for 7 days. CFU per gram of gastric tissue was determined.

Gastritis Assessment

H. pylori and *H. felis* gastritis was assessed using the modified Sydney grading system for gastritis (19). Longitudinal

strips, starting from the squamocolumnar junction to the gastroduodenal junction, taken from the greater curvature of the stomach were fixed in 10% formalin and processed by standard methods, embedded in paraffin, cut at a thickness of 4 μm, and stained with hematoxylin and eosin. The glandular mucosa of the body, transitional zone, and antrum were examined blindly by two independent observers. Lamina propria, submucosa, muscularis propria, and serosa of the three gastric regions were assessed for acute and chronic inflammatory cell infiltrate. The severity of the infiltrate was graded as 0 for no inflammation, 1 for occasional inflammatory cells, 2 for multifocal inflammatory infiltrate, 3 for discontinuous band of inflammation, and 4 for continuous band of inflammation. Glandular neutrophil infiltrate was assessed and graded as 0 for none, 1 for intraepithelial infiltration, 2 for infiltration into crypts, and 3 for crypt destruction. In addition, the number of lymphoid aggregates from each strip was counted. The presence (graded 1) or absence (graded 0) of change in architecture, cystic changes, loss of specialized cells, and intestinal metaplasia were assessed. Gastric mucosal thickness was also measured.

Measurement of Surface Hydrophobicity

Twenty-six C57BL/6 mice were used. Sixteen mice were infected with the SS1 strain of *H. pylori* for 4 weeks. Ten mice were not infected. Eight of the infected mice and five noninfected mice were gavaged with NAC, 40 mg daily for 10 days; the remaining half of each group received water. Mice were sacrified on day 11. The stomach was removed and two 3-mm^2 pieces of body and one 3-mm^2 piece of antral mucosa were used for contact angle measurement. *H. pylori* status of the mice was determined by culturing *H. pylori* from gastric tissues.

Surface hydrophobicity was determined by measuring the contact angle. This is based on the principle that when a liquid drop is applied to a solid surface, a contact angle is formed as a result of the equilibrium of surface forces at the triple point of the air–liquid–solid interface. The contact angle has an inverse relationship to the surface free energy of the solid. With the surface tension of the liquid remaining the same, the contact angle will be lower as the liquid surface tension is closer to the surface energy of the solid. Therefore the surface energy or hydrophobicity of the solid surface can be derived from the contact angle (20).

A 1 μl droplet of distilled water was applied to the surface of gastric mucosa. A digital image was taken using a Sony high-resolution B&W videocamera attached to a Macintosh Centris 660AV computer with an internal frame grabber. The contact angle was measured using National Institutes of Health image analysis program version 1.61. A large angle reflects a high surface hydrophobicity (21).

Statistics

H. pylori loads were compared between NAC and sham-treated mice using the SPSS independent sample Student *t* test. Contact angle measurements from four groups of mice were analyzed using SPSS one-way ANOVA with LSD post hoc test. Gastritis scores were compared using Mann–Whitney analysis of nonparametric data. The number of lymphoid aggregates and gastric wall thickness were compared between NAC and sham-treated mice using the SPSS independent sample Student *t* test. A P value <0.05 was considered significant.

RESULTS

NAC Inhibited the Growth of *H. pylori* Strain SS1 In vitro

NAC inhibition of the growth of *H. pylori* strain SS1 was observed using the agar dilution susceptibility test (Figure 1) and confirmed by the broth dilution susceptibility test (Figure 2). The MIC of NAC for *H. pylori* strain SS1 was between 5 and 10 mg/ml of NAC using the agar dilution susceptibility test (Figure 1) and 10–15 mg/ml of NAC using the broth dilution method (Figure 2). The growth of SS1 strain was completely inhibited only after 3 days of exposure to NAC at 15 mg/ml, suggesting that NAC is bacteristatic against *H. pylori*. The inhibitory effect of NAC was independent of the pH of the medium. Both the broth and the agar containing the various concentrations of NAC had similar pH's (data not shown).

NAC Reduced the *H. pylori* Load in Mice Infected with *H. pylori* Strain SS1

Mice were infected for 4 months with *H. pylori* strain SS1. The *H. pylori* load in mice treated with NAC (120 mg per day, 14 days) was reduced by almost 1 log compared with mice receiving sham treatment ($P = 0.05$) (Figure 3). Four mouse stomach homogenates from the NAC group and three from the controls were contaminated and were excluded from analysis. No *H. pylori* growth was detected in stomach homogenates from three other mice receiving NAC. We are uncertain whether *H. pylori* eradication in these mice was due to NAC. Eradication is unusual for a monotherapy, and results from these three mice were inconsistent with those from the remaining 13 NAC-treated mice, therefore we elected to exclude the results of these three mice from analysis, although this does not preclude a treatment effect in isolated mice.

Pretreatment with NAC Also Reduced the *H. Pylori* Load in Infected Mice but Did Not Prevent *H. Pylori* Colonization

Mice were treated with NAC (40 mg/day) for 3 days prior to being infected with *H. pylori* strain SS1. NAC treatment then was continued for a further 7 days. Control mice received sham treatment with water. *H. pylori* load in NAC-treated mice was significantly reduced compared to that in control mice ($P < 0.01$) (Figure 4). However, pretreatment with NAC did not alter the gastric microenvironment sufficiently to prevent *H. pylori* colonization.

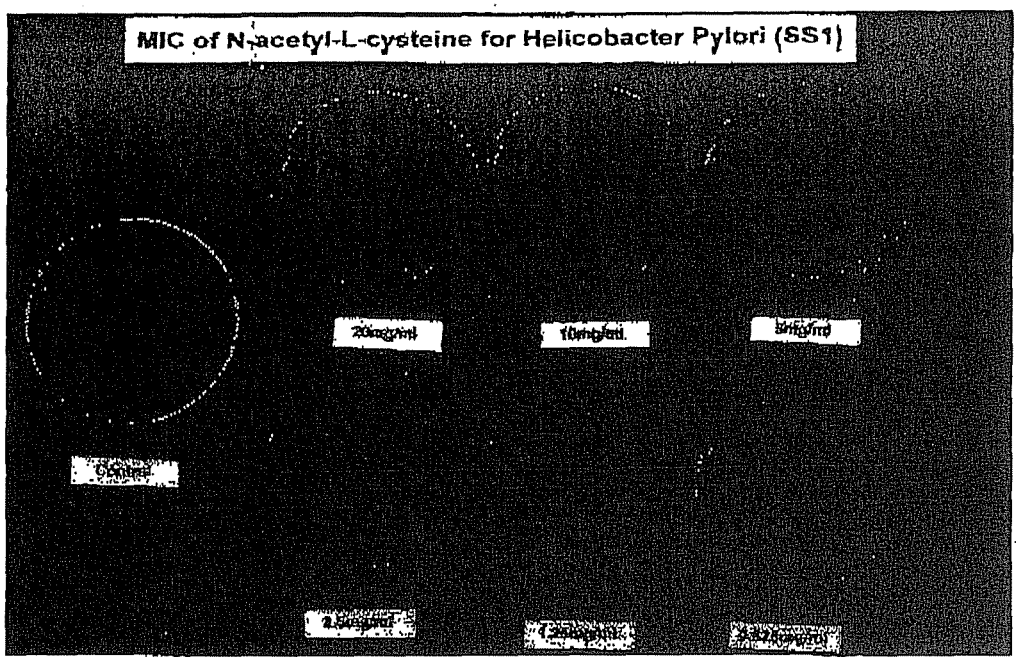

Fig 1. Agar susceptibility test demonstrating that NAC inhibited *H. pylori* growth. *H. pylori* strain SS1 growth was observed in the control agar plate and in plates containing up to 5 mg/ml NAC. No growth was detected in agar plates containing 10 and 20 mg/ml NAC. This indicates that the minimal inhibitory concentration (MIC) of NAC for strain SS1 is between 5 and 10 mg/ml, $n = 2$.

Both *H. Pylori* Infection and NAC Reduced the Surface Hydrophobicity of Murine Gastric Mucosa

NAC is known to be a mucolytic agent that can alter the biophysical properties of mucus. Surface hydrophobicity was determined by measuring the droplet contact angle. Contact angle measurements from gastric mucosa of mice treated with NAC (40 mg/day, 10 days) were significantly lower compared with mice receiving sham treatment with water (Figure 5). This indicates that NAC directly alters the surface properties of the gastric mucosa by reducing

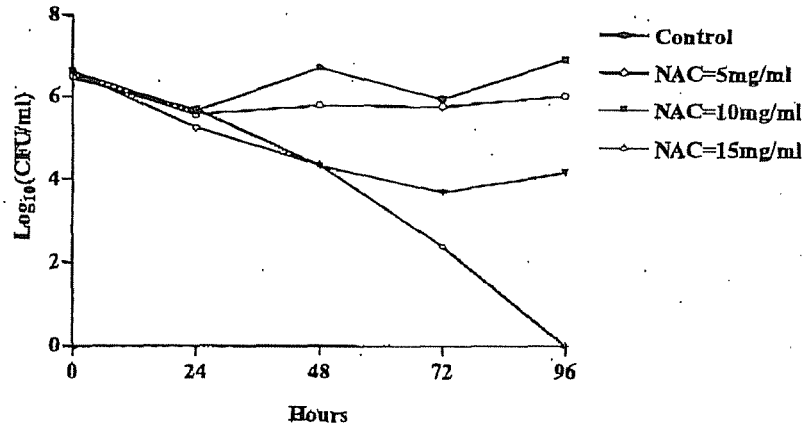

Fig 2. Broth susceptibility test demonstrated that NAC inhibited *H. pylori* growth. *H. pylori* strain SS1 growth was maintained in control broth and broth containing 5 mg/ml NAC. SS1 growth was suppressed by 2 $\log_{10}$ in broth containing 10 mg/ml NAC after 48 hr of growth. No growth of SS1 was detected in broth containing 15 mg/ml NAC at 96 hr of growth. The minimal inhibitory concentration of NAC using the broth dilution method is between 10 and 15 mg/ml, $n = 1$.

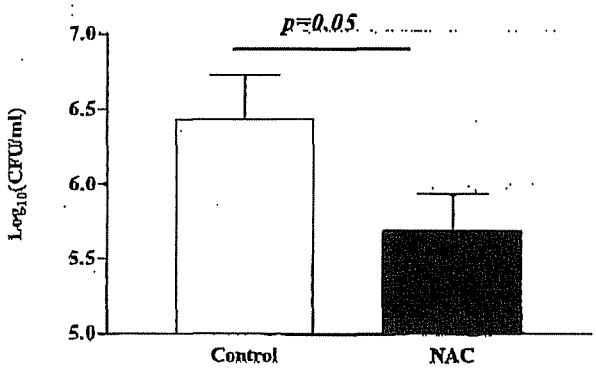

Fig 3. Treatment with NAC reduced the *H. pylori* strain SS1 load in mice. Both groups of mice were infected with strain SS1 for 4 months. The white column represents the mean SS1 load in 17 mice treated with water. The black column represents the mean SS1 load in 13 mice treated with NAC (120 mg/day for 14 days). Bars represent mean +/− SE. Student *t* test, $P = 0.05$.

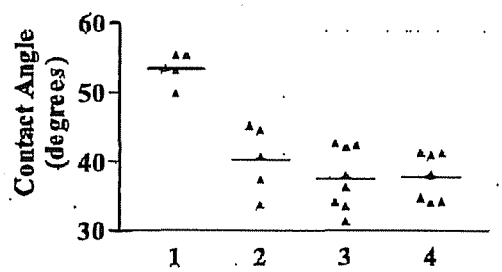

Fig 5. NAC and *H. pylori* infection both reduced the surface contact angle of mouse gastric mucosa. Group 1 represents normal mice ($n = 5$), group 2 represents normal mice treated with NAC (40 mg/day for 10 days; $n = 5$), group 3 represents mice infected with strain SS1 for 4 weeks ($n = 8$), and group 4 represents SS1-infected mice treated with NAC (40 mg/day for 10 days; $n = 7$). The mean contact angle of group 1 was significantly higher than those of groups 2, 3, and 4. One-way ANOVA, $P < 0.01$.

mucosal surface hydrophibicity. Mucosal contact angle measurements for mice infected with *H. pylori* strain SS1 for 4 weeks were also significantly lower than for noninfected mice (Figure 5). This suggests that *H. pylori* infection alone reduced the surface hydrophobicity of the infected gastric mucosa. However, gastric mucosal contact angle measurements from *H. pylori* strain SS1-infected mice (4 weeks) treated with NAC (40 mg/day, 10 days) did not decrease further compared with mucosal contact angle measurements in *H. pylori* infected mice or NAC-treated noninfected mice (Figure 5). These results suggested that NAC did not reduce further the surface hydrophobicity of *H. pylori*-infected mice. Thus any effect of NAC on *H. pylori*-infected mice cannot be explained entirely by the alteration in the electrostatic properties of mucus.

NAC Treatment Did Not Affect Gastric Inflammation in *H. felis*-Infected Mice

Minimal inflammation was seen in C57BL/6 mice infected with *H. pylori* in the short term. Therefore a mouse gastritis model was used which required infection with *H. felis*. Twenty-one C57BL/6 mice were infected with *H. felis* for 12 weeks. Eleven mice were treated with NAC (40 mg/day for 14 days) and 10 mice were sham treated with water via gavage. No significant differences in both chronic lymphocytic and granulocytic inflammatory infiltrates and epithelial alteration such as parietal cell loss in the gastric mucosa were observed between the NAC and the sham-treated groups of mice (Figure 6). There were also no significant differences observed in the number of

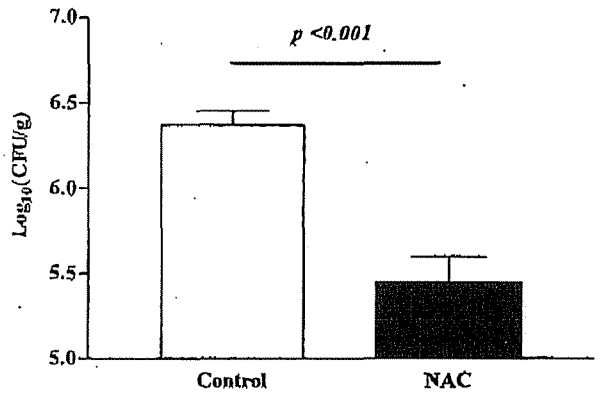

Fig 4. Pretreatment with NAC reduced the *H. pylori* strain SS1 load in mice. Both groups of mice were infected for 1 week. The white column represents the mean *H. pylori* load in 20 mice sham treated with water prior to and during infection for a total of 10 days. The black column represents the mean *H. pylori* load in 15 mice treated with NAC (40 mg per day for 10 days). Bars represent mean +/− SE. Student *t* test, $P < 0.001$.

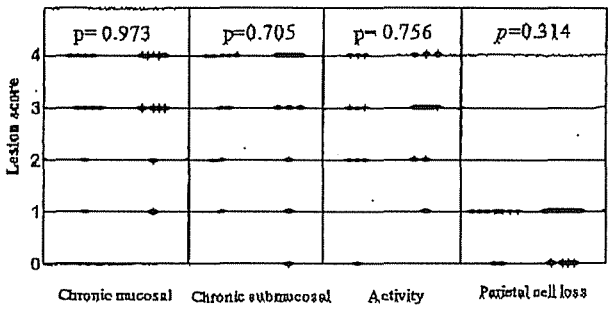

Fig 6. NAC did not alter the gastric inflammatory score in *H. felis*-infected mice. Circles represent mice infected with *H. felis* and treated with water; diamonds represent mice infected with *H. felis* and treated with NAC (40 mg/day for 14 days). The number of symbols indicates the number of mice obtaining that score. After 12 weeks of infection with *H. felis*, mice treated with NAC showed no difference in their gastric corpus chronic (lymphocytic) infiltrate, active (granulocytic) infiltrate, and parietal cell loss in comparison to infected mice treated with water.

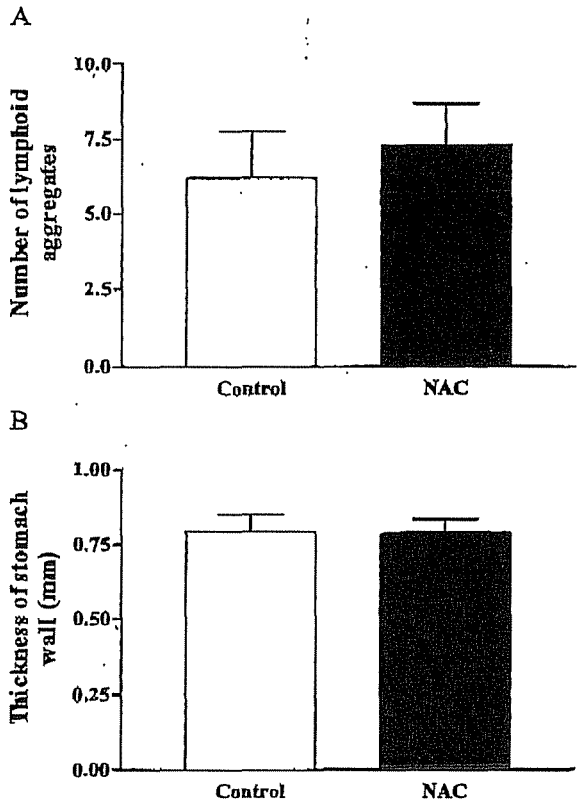

Fig 7. (A) There was no difference in the number of lymphoid aggregates seen per longitudinal strip of gastric tissue between water ($n = 10$)- and NAC (40 mg/day for 14 days; $n = 11$)-treated *H. felis*-infected mice. (B) The gastric wall thickness in the two groups of mice was similar.

lymphoid aggregates and gastric wall thickness between the two groups (Figures 7A and 7B). These results imply that although NAC does not improve gastritis in *H. felis*-infected mice, at least it does not worsen *H. felis*-induced gastritis.

DISCUSSION

This study has demonstrated that *N*-acetylcysteine (NAC) is capable of inhibiting the growth of *H. pylori* both in vitro and in vivo. NAC dose dependently inhibits the growth of *H. pylori* in both agar and broth susceptibility tests. In the mouse model, NAC significantly reduces the *H. pylori* load in the gastric mucosa. Our finding is also supported by Zala *et al.*, who showed that NAC at 1.2 g twice a day for 10 days improved the eradication of *H. pylori* in smokers who were concomitantly treated with omeprazole and amoxycillin (22). NAC also changes the physicochemical properties of the gastric mucus as demonstrated by the reduction in surface hydrophobicity of mouse gastric mucosa treated with NAC. However, NAC does not alter the underlying gastric inflammation induced by *H. pylori* or *H. felis*. We elected to use a lower dose of NAC and shorter length of treatment in the prevention trial in order to reduce the number of gavages these mice received. A reduction in the *H. pylori* load was observed with the prevention trial protocol and therefore the higher NAC dose and longer length of treatment used in the treatment trial were not tested in subsequent experiments.

The antibacterial effect of NAC was previously shown against both gram-positive and gram-negative microorganisms, especially *Pseudomonas aeruginosa*, and thought to be predominantly bacteristatic (11). Recently, NAC has been shown to inhibit bacterial biofilm formation (23, 24). NAC has also been shown to reduce adhesion of *Streptococcus pneumoniae*, *Hememophilus influenza*, and *Moraxella catarrhalis* in pharyngeal epithelial cells (12, 25). Extracellular polysaccharides (EPS), a major component in biofilms, are produced in large quantities by bacteria to bind biofilm into a matrix and anchor the biofilm to the surface (26). EPS production is reduced in the presence of NAC (23). *H. pylori* has been demonstrated to form a water-insoluble biofilm in tissue culture and this phenomenon is likely to occur in vivo (27). In addition to its antimicrobial properties, NAC's ability to inhibit biofilm formation, EPS production, and adhesion of bacteria to the mucosal surface may contribute to the reduced *H. pylori* load demonstrated in our study.

The mucus layer overlying the gastric mucosa is an integral part of the stomach, providing protection, lubrication, and a medium for transport between the epithelial layer and the luminal content. The mucus layer is composed predominantly of mucin glycoproteins that are produced by the mucus cells contained within the gastric glands. The viscosity and elasticity of mucus are derived primarily from these mucins. Bacteria capable of colonizing the gastric mucus layer derive significant ecological advantages from this layer, such as nutrition, avoidance of expulsion by gastric peristalsis, and protection from the harsh acid luminal content of the stomach. It is now well established that bicarbonate secretion neutralizes luminal acid within the matrix of the mucus layer, forming a pH gradient across the mucus layer, with a high pH at the base of the mucus layer nearest to the epithelium and a low pH at the top, where the mucus makes direct contact with the acidic luminal content (28). Therefore it is not surprising that *H. pylori* takes advantage of this pH gradient and colonizes most abundantly in the basal mucus gel layer, thus protecting itself from the low pH of the gastric content. *H. pylori* colonization is also associated with disruption of the multilaminated mucus layer and formation of vacuoles

(29). *H. pylori* has been shown to alter mucus synthesis and secretion and mucin gene expression (30). *H. pylori* LPS initially increases mucin production in gastric biopsies, however, prolonged exposure results in decreased mucin production and synthesis. *H. pylori* suppresses expression of the MUC1 and MUC5AC genes in human gastric cell lines (31).

Many agents tested in our laboratory and others including desferoxamine (32), nutriceuticals, and probiotics (33) inhibit *H. pylori* growth in vitro but rarely also inhibit growth in vivo. In our study, NAC inhibited *H. pylori* growth in both settings. Whether NAC's mucolytic property, *i.e.*, its ability to cause dissipation of the pH gradient and to reduce the thickness of the mucus gel layer (34), contributed to the reduction in the *H. pylori* load in mice was not confirmed by our hydrophobicity studies. The mucolytic property of NAC on gastric mucosa was demonstrated by the significantly reduced mucosal surface hydrophobicity of mice treated with NAC in comparison to controls. Mucosa obtained from *H. pylori*-infected mice also showed a significant reduction in surface hydrophobicity in comparison to those from noninfected mice. These results are consistent with previously published work (35). However, the reduction in surface hydrophobicity with NAC treatment and *H. pylori* infection was not synergistic as shown by the lack of further reduction in mucosal surface hydrophobicity in infected mice treated with NAC in comparison to untreated infected mice. Although we were not able to confirm the mucolytic property of NAC contributing to the reduced *H. pylori* load in mice, Gotoh *et al.* demonstrated, using pronase as a mucolytic agent, a further improvement in the efficacy of triple eradication therapy against *H. pylori*, from a cure rate of 76% to one of 94%. Pronase was shown to have poor antimicrobial activity against *H. pylori* but was able to reduce the thickness of the surface mucus gel layer in humans (36).

Oxidative stress plays a role in *H. pylori*-induced mucosal damage; the migration of inflammatory cells to gastric mucosa is followed by a respiratory burst liberating superoxide and other reactive oxygen metabolites (37,38). These free radicals can initiate lipid peroxidation and DNA damage leading to cellular destruction (39–41). Therefore the balance between the generation of free radicals and the production of endogenous as well as exogenous antioxidants is critical to the physiological function of cells. *H. pylori* eradication has been shown to attenuate oxidative stress in human gastric mucosa (42). Antioxidant enzymes such as superoxide dismutase (a well-known scavenger of superoxide radicals) are up-regulated in *H. pylori* infection to protect cells against oxidative damages (43). Exogenous antioxidants such as ascorbic acid have been shown to reduce the risk for gastric disease and cancer in some epidemiological studies (8). High-dose ascorbic acid inhibits the growth of *H. pylori* both in vitro and in vivo as tested in Mongolian gerbils and humans (7, 44). Astaxanthin, a lipid-soluble antioxidant found in seafood, lowers the *H. pylori* level of colonization and gastric inflammatory score in mice together with the level of lipid peroxidation (45). NAC, an antioxidant with a precursor of glutathione and a scavenger of hydroxyl radicals (46), has been shown to inhibit *H. pylori*-induced activation of nuclear factor kappa B (NF-kB) activation, a critical transcription factor for proinflammatory cytokines in gastric epithelial cells (47). It remains controversial whether NAC's inhibition of NF-kB is secondary to its scavenging of reactive oxygen radicals (48). NAC potentially could reduce the level of *H. pylori* colonization as well as the degree of gastric inflammation via its antioxidant properties and its ability to inhibit NF-kB activation. In our study, it is possible that NAC's antioxidant properties contribute to the reduction in the *H. pylori* load in NAC-treated mice. We were not able to demonstrate a reduction in gastric inflammation in our *H. pylori*-infected C57BL/6 mice treated with NAC. However, minimal gastritis was observed in C57BL/6 mice infected with *H. pylori*, making assessment of gastritis less accurate. Therefore a mouse gastritis model infected with *H. felis*, which causes a much more severe degree of gastritis in C57BL/6 mice, was also employed (49). Our data using the *H. felis* gastritis model did not demonstrate that NAC treatment reduces the severity of *Helicobacter*-induced gastritis. One possible explanation is the short duration for which these mice were exposed to NAC treatment.

With the rise in the prevalence of metronidazole and clarithromycin resistance of *H. pylori* clinical isolates (5), NAC could potentially be a new class of medication employed to eradicate *H. pylori*. This study demonstrates the conceptual use of a mucolytic/antioxidant agent in the treatment of this very common infection. Potentially NAC could be used in combination with triple therapy in cases where *H. pylori* is resistant to standard treatment.

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