Published in final edited form as:
*J Clin Gastroenterol*. 2011 ; 45(10): 841–843. doi:10.1097/MCG.0b013e31822be4d6.

# The Potential Role of *N*-Acetylcysteine for the Treatment of *Helicobacter pylori*

**Kian Makipour, MD** and **Frank K. Friedenberg, MD, MS (Epi)**
Gastroenterology Section, Temple University School of Medicine, Philadelphia, PA

Resistance to *Helicobacter pylori* has become increasingly common with triple or quadruple therapy with cure rates of approximately 80%. The success of therapy is thought to be influenced by patient compliance, medication side effects, and antibiotic resistance.[1–3] A treatment failure rate of 10% to 20% has driven investigators to seek alternative modes of therapy.[1] *N*-acetylcysteine (NAC), which is capable of destroying bacterial biofilm, is an emerging treatment for recalcitrant infections.

*H. pylori* colonizes the human stomach by overcoming gastric acidity and peristalsis, and circumventing the host's immune response.[3] The enzyme urease is used to overcome gastric acidity whereas 5 to 6 flagella allow the organism to oppose the host's peristalsis.[4–6] Proinflammatory molecules produced by the host such as nitric oxide result in bactericidal activity.[7] *H. pylori* express the enzyme arginase to direct arginine away from inducible nitric oxide synthase and limit the production of nitric oxide.[7] In addition, *H. pylori* lipopolysaccharide and flagellin are anergic allowing them to evade activation of toll-like receptors and innate immunity.[8,9] Recent studies suggest biofilm formation plays an important role in *H. pylori* colonization and resistance to antibacterial therapy.[10]

The production of biofilm by *H. pylori* likely facilitates antibiotic resistance similar to many other bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus epidermis*, and *Escherichia coli*.[11–13] Biofilm is composed of an extracellular matrix and bacteria. The extracellular matrix is a complex of hydrated polyanionic exopolysaccharides such as polymerized β-1,6-*N*-acetyl-D-glucosamine, cellulose, the branched polymer colanic acid, protein, and DNA of bacterial origin.[14,15] Gram-negative bacteria share several processes in the formation of biofilm.[14] Biofilm formation begins with the binding of bacteria to an extracellular surface through flagella or pili.[8,9,14,16] Commonly, binding occurs on the surface of medical devices implanted in the host. Bacteria can sense the availability of nutrients in their environment.[14,17] Some bacteria form biofilm in nutrient-poor environments whereas others require nutrient-rich environments.[14] For example, *Vibrio cholera* biofilm formation is induced by glucose.[14] Glucose is sensed by the phosphoenoylpyruvate phosphotransferase system that transfers a phosphate moiety to sugars resulting in activation of exopolysaccharide gene transcription and biofilm formation.[14] The cyclic adenosine monophosphate (cAMP)- cyclic AMP receptor protein complex can both induce and repress biofilm formation.[14] The cAMP receptor protein complex upregulates biofilm formation by turning off the repressor HapR and turning on the activator VpsR.[14] Conversely, the cAMP receptor protein complex downregulates biofilm formation by turning off diguanylate cyclase CdgA.[14]



Reprints: Frank K. Friedenberg, MD, Temple University Hospital, 3401 North Broad Street, Philadelphia, PA 19140 (frank.friedenberg@temple.edu).

The authors declare that they have nothing to disclose.

Organic and inorganic molecules can serve as regulators of biofilm formation.[14] For example, indole induces biofilm formation in many Gram-negative bacteria by serving as a source of carbon in nutrient-poor environments.[14] Indole is formed by the hydrolysis of tryptophan by the enzyme tryptophanase.[14] In addition, polyamines are essential for cell growth by serving as metabolic signals.[14] In *V. cholera*, the polyamine norspermidine activates biofilm formation.[14] Similarly, inorganic molecules (eg, iron) can serve as positive regulators of biofilm formation.[14] Host-derived molecules such as bile promotes biofilm formation in certain bacteria such as *V. cholera.*[14]

Bacteria within biofilm communicate with each other by forming quarom-sensing circuits.[14,15,17] These bacteria release or secrete autosignals that are detected by their neighbors to either upregulate or downregulate growth.[14–17] One such system that is common in Gram-negative bacteria is the *luxI/luxR* system that activates gene expression.[14,18]

There are 3 important mechanisms which facilitate the survival of organisms that produce biofilm. Biofilm reduces antibiotic penetration, decreases the growth of microbial cells, and allows for the differentiation of cells into protected phenotypes.[11,15,17] Biofilms consist of a thick exopolysaccharide matrix coating a surface which can be liquid or solid (living cells or devices).[11,15,17] These matrices contain molecules that deactivate antibiotics at a rate faster than the antibiotic's diffusion capacity.[11,15,17] Also, the growth rate of cells within a biofilm varies. Cells in the deeper layers, where waste accumulates and nutrients are scarce, enter a quiescent phase.[11,15,17] These slow-growing bacterial cells are more difficult to eradicate than their faster-growing counterparts. Lastly, a subpopulation of cells in biofilm differentiates into protected phenotypes and passes on their resistance traits to their neighboring cells.[11,15,17]

Three important studies have demonstrated that *H. pylori* forms biofilm. First, Stark et al[19] showed that when *H. pylori* is grown in a glass fermenter, biofilm formation occurs at the interface of air and liquid. Subsequently, Mackay et al[20] showed that *H. pylori* is able to bind to mixed species biofilm grown on the interface of air and a steel surface. More recently, Carron et al,[21] used scanning electron microscopy of gastric biopsies obtained during endoscopy, demonstrated the presence of biofilms on *H. pylori*-positive specimens.

Relatively little is known in regards to the process of biofilm formation by *H. pylori*.[4] *H. pylori* adheres to sulfated oligosaccharides, glycolipids, and the fucosylated blood group oligosaccharide Lewis[b] on the gastric epithelium using flagella.[22,23] *Lux* is a quarom-sensing system possessed by *H. pylori*.[18,24] Activation of a *lux* encoded autoinducer leads to the production and detection of signaling molecules which allow organisms to communicate with each other and detect information about their external environment and cell density.[24] The *cag* type IV secretion system is essential for *H. pylori* biofilm formation.[24] VirB proteins are involved in the assembly of bacterial membranes. VirD4 guides CagA into the transport channel and after translocation CagA is tyrosine phosphorylated.[14,25] Phosphorylation induces actin cystoskeletal changes and expression of the scattering or hummingbird phenotype allowing the bacteria to splay out over a surface.[14,25]

Several studies have demonstrated a role for NAC in destroying biofilm due to its mucolytic properties.[26–28] NAC acts as a mucolytic agent by cleaving disulfide bonds which crosslink glycoproteins.[29] NAC is also bacteriostatic. In an in vitro study by Parry and Neu,[28] NAC was found to inhibit the growth of both Gram-negative and Gram-positive microorganisms. Inoculum size and dose administered greatly affected the ability of NAC to inhibit bacterial growth. Perez-Giraldo et al[12] used spectrophotometry to quantify the formation of biofilms by *S. epidermis* in the presence of NAC. Biofilm diminished significantly as the

concentration of NAC increased. Olofsson et al,[27] demonstrated the utility of NAC in reducing biofilm formation, though more so by Gram-positive than by Gram-negative strains of bacteria. Moreover, in this study, NAC was shown to reduce polysaccharide production which is an important component of biofilms.[27] In addition, Zhao and Liu[13] demonstrated disruption of *P. aeruginosa* biofilms beginning at a NAC concentration of 0.5 mg/mL with maximal effect at a concentration of 10 mg/mL. NAC concentrations of 0.5 and 1 mg/mL decreased polysaccharide production by 27.64% and 44.59%, respectively.[13]

Conventional first line *H. pylori* therapy consists of triple therapy followed by quadruple therapy in patients who fail to eradicate *H. pylori*. It is hypothesized that *H. pylori* biofilm contributes to failure of triple or quadruple therapy in a substantial number of patients. Recent studies suggest NAC has utility in *H. pylori* biofilm eradication. First, Zala et al[30] conducted a prospective, randomized clinical trial to investigate whether the addition of NAC to omeprazole/amoxicillin increases eradication. Thirty-four subjects with duodenal ulcers and biopsy proven *H. pylori* was included. They underwent ulcer therapy with omeprazole 20 mg PO daily from day 1 to day 20. They then underwent eradication therapy with omeprazole 40 mg PO BID and amoxicillin 750mg PO daily for 10 days. Those in group A also received NAC 600mg PO daily.[30] Among subjects in group A, *H. pylori* was successfully eradicated in 12/17 (71%). Among subjects in group B, *H. pylori* was eradicated in only 7/17 (41%). Interestingly, cigarette smoking was associated with therapeutic failure in group B only.

A study in 2005 by Gurbuz et al[31] included 58 *H. pylori*-positive patients. Participants were divided into 2 groups. Both groups received 10 days of oral clarithromycin 500mg BID and lansoprazole 30 mg BID. Group 1 also received 10mL (400 mg) of NAC TID. Patients underwent endoscopy 1 month after treatment to assess eradication. In group 1, 14/28 (50%) patients were successfully treated whereas 7/30 (23.3%) of the controls achieved eradication ($P$=0.034). The authors postulated that NAC augments the activity of dual therapy by reducing the thickness of biofilm.[31]

Recently, a study in Italy by Cammarota et al[32] randomized 40 patients who had previously failed *H. pylori* treatment to either receive NAC 600 mg PO daily for 7 days followed by culture-guided *H. pylori* therapy (group 1) or culture-guided *H. pylori* therapy alone (group 2). *H. pylori* eradication was assessed by a urea breath test. During endoscopy, before treatment, 2 gastric biopsies were used to image biofilm by scanning electron microscopy whereas 2 additional biopsies were used for *H. pylori* culture, antibiotic susceptibility testing, and genetic analysis. An in vitro arm included gastric biopsies from 10 patients who had previously failed *H. pylori* therapy. Biopsies from 5 of these patients were cultured with NAC (2 mg/mL) whereas biopsies from the remaining 5 patients were cultured without NAC and tested for the presence of biofilm. The cultures without NAC were later tested with increasing concentrations of NAC to assess its effect on biofilm. Thirteen of 20 patients (65%) in group 1 were successfully treated while only 4 of 20 (20%) patients in group 2 eradicated *H. pylori* ($P$<0.01). In vitro, 0 of 5 specimens cultured with NAC generated biofilm whereas 5 of 5 specimens cultured without NAC developed biofilm. Among the specimens cultured without NAC, adding increasing concentrations of NAC reduced the amount of biofilm. In short, the authors concluded that pretreatment with NAC followed by antibiotic therapy improves treatment outcomes by eradication of biofilm.[32]

## CONCLUSIONS

In conclusion, *H. pylori* organisms are able to evade host defenses and resist antibiotic treatment leading to persistent infection. One reason for the high rate of treatment failure is biofilm formation. Biofilm deactivates antibiotics at a faster rate than their diffusion

capacity, slows the metabolic activity of cells in the deeper layers leading to a quiescent state, and favors the propagation of a protected phenotype. Several studies have demonstrated the efficacy of NAC in the eradication of biofilm. NAC cleaves disulfide bonds which crosslink glycoproteins in biofilm. Recently, several studies have suggested that NAC has efficacy in the treatment of resistant *H. pylori* infections. Pretreatment with NAC followed by antibiotic therapy has an additive effect in the patients with resistant infection. Future studies are needed to further define the role of NAC in the treatment of *H. pylori* infection.

## Acknowledgments

F.K.F. was awarded K24DK083268.

## References

1. Bytzer P, O'Morain C. Treatment of *Helicobacter pylori*. Helicobacter. 2005; 10(Suppl 1):40–46. [PubMed: 16178970]
2. Ford AC, Delaney BC, Forman D, et al. Eradication therapy in *Helicobacter pylori* positive peptic ulcer disease: systematic review and economic analysis. Am J Gastroenterol. 2004; 99:1833–1855. [PubMed: 15330927]
3. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med. 2002; 347:1175–1186. [PubMed: 12374879]
4. Skouloubris S, Thiberge JM, Labigne A, et al. The *Helicobacter pylori* UreI protein is not involved in urease activity but is essential for bacterial survival in vivo. Infect Immun. 1998; 66:4517–4521. [PubMed: 9712811]
5. Eaton KA, Krakowka S. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect Immun. 1994; 62:3604–3607. [PubMed: 8063376]
6. Eaton KA, Suerbaum S, Josenhans C, et al. Colonization of gnotobiotic piglets by *Helicobacter pylori* deficient in two flagellin genes. Infect Immun. 1996; 64:2445–2448. [PubMed: 8698465]
7. Gobert AP, McGee DJ, Akhtar M, et al. *Helicobacter pylori* arginase inhibits nitric oxide production by eukaryotic cells: a strategy for bacterial survival. Proc Natl Acad Sci U S A. 2001; 98:13844–13849. [PubMed: 11717441]
8. Gewirtz AT, Yu Y, Krishna US, et al. *Helicobacter pylori* flagellin evades toll-like receptor 5-mediated innate immunity. J Infect Dis. 2004; 189:1914–1920. [PubMed: 15122529]
9. Lee SK, Stack A, Katzowitsch E, et al. *Helicobacter pylori* flagellins have very low intrinsic activity to stimulate human gastric epithelial cells via TLR5. Microbes Infect. 2003; 5:1345–1356. [PubMed: 14670447]
10. Yonezawa H, Osaki T, Kurata S, et al. Assessment of in vitro biofilm formation by *Helicobacter pylori*. J Gastroenterol Hepatol. 2010; 25(suppl 1):S90–S94. [PubMed: 20586874]
11. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284:1318–1322. [PubMed: 10334980]
12. Perez-Giraldo C, Rodriguez-Benito A, Moran FJ, et al. Influence of N-acetylcysteine on the formation of biofilm by *Staphylococcus epidermidis*. J Antimicrob Chemother. 1997; 39:643–646. [PubMed: 9184365]
13. Zhao T, Liu Y. N-acetylcysteine inhibit biofilms produced by *Pseudomonas aeruginosa*. BMC Microbiol. 2010; 10:140. [PubMed: 20462423]
14. Karatan E, Watnick P. Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev. 2009; 73:310–347. [PubMed: 19487730]
15. del Pozo JL, Patel R. The challenge of treating biofilm-associated bacterial infections. Clin Pharmacol Ther. 2007; 82:204–209. [PubMed: 17538551]
16. Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother. 2001; 45:999–1007. [PubMed: 11257008]

17. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001; 358:135–138. [PubMed: 11463434]
18. Joyce EA, Bassler BL, Wright A. Evidence for a signaling system in *Helicobacter pylori*: detection of a luxS-encoded autoinducer. J Bacteriol. 2000; 182:3638–3643. [PubMed: 10850976]
19. Stark RM, Gerwig GJ, Pitman RS, et al. Biofilm formation by *Helicobacter pylori*. Lett Appl Microbiol. 1999; 28:121–126. [PubMed: 10063642]
20. Mackay WG, Gribbon LT, Barer MR, et al. Biofilms in drinking water systems: a possible reservoir for *Helicobacter pylori*. J Appl Microbiol. 1998; 85(suppl 1):52S–59S. [PubMed: 21182693]
21. Carron MA, Tran VR, Sugawa C, et al. Identification of *Helicobacter pylori* biofilms in human gastric mucosa. J Gastrointest Surg. 2006; 10:712–717. [PubMed: 16713544]
22. Boren T, Falk P, Roth KA, et al. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science. 1993; 262:1892–1895. [PubMed: 8018146]
23. Simon PM, Goode PL, Mobasseri A, et al. Inhibition of *Helicobacter pylori* binding to gastrointestinal epithelial cells by sialic acid-containing oligosaccharides. Infect Immun. 1997; 65:750–757. [PubMed: 9009338]
24. Cole SP, Harwood J, Lee R, et al. Characterization of monospecies biofilm formation by *Helicobacter pylori*. J Bacteriol. 2004; 186:3124–3132. [PubMed: 15126474]
25. Selbach M, Moese S, Meyer TF, et al. Functional analysis of the *Helicobacter pylori* cag pathogenicity island reveals both VirD4-CagA-dependent and VirD4-CagA-independent mechanisms. Infect Immun. 2002; 70:665–671. [PubMed: 11796597]
26. Millea PJ. N-acetylcysteine: multiple clinical applications. Am Fam Physician. 2009; 80:265–269. [PubMed: 19621836]
27. Olofsson AC, Hermansson M, Elwing H. N-acetyl-L-cysteine affects growth, extracellular polysaccharide production, and bacterial biofilm formation on solid surfaces. Appl Environ Microbiol. 2003; 69:4814–4822. [PubMed: 12902275]
28. Parry MF, Neu HC. Effect of N-acetylcysteine on antibiotic activity and bacterial growth in vitro. J Clin Microbiol. 1977; 5:58–61. [PubMed: 401831]
29. Dodd S, Dean O, Copolov DL, et al. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. Expert Opin Biol Ther. 2008; 8:1955–1962. [PubMed: 18990082]
30. Zala G, Flury R, Wust J, et al. Omeprazole/amoxicillin: improved eradication of *Helicobacter pylori* in smokers because of N-acetylcysteine. Schweiz Med Wochenschr. 1994; 124:1391–1397. [PubMed: 8091167]
31. Gurbuz AK, Ozel AM, Ozturk R, et al. Effect of N-acetyl cysteine on *Helicobacter pylori*. South Med J. 2005; 98:1095–1097. [PubMed: 16351030]
32. Cammarota G, Branca G, Ardito F, et al. Biofilm demolition and antibiotic treatment to eradicate resistant *Helicobacter pylori*: a clinical trial. Clin Gastroenterol Hepatol. 2010; 8:817–820. e3. [PubMed: 20478402]