

Efficacy of Sulforaphane in Eradicating *Helicobacter pylori* in Human Gastric Xenografts Implanted in Nude Mice

Xavier Haristoy,¹ Karine Angioi-Duprez,² Adrien Duprez,³ and Alain Lozniewski^{1*}

Laboratoire de Bactériologie-Virologie, UMR CNRS 75-65,¹ Laboratoire de Microchirurgie Expérimentale,² and Laboratoire d'Anatomie Pathologique EMI INSERM 0014,³ Faculté de Médecine, Université Henri-Poincaré, Vandœuvre-les-Nancy, France

Received 15 November 2002/Returned for modification 24 May 2003/Accepted 25 August 2003

Sulforaphane, an isothiocyanate abundant in the form of its glucosinolate precursor in broccoli sprouts, has shown in vitro activity against *Helicobacter pylori*. We evaluated the effect of sulforaphane in vivo against this bacterium by using human gastric xenografts in nude mice. *H. pylori* was completely eradicated in 8 of the 11 sulforaphane-treated grafts. This result suggests that sulforaphane might be beneficial in the treatment of *H. pylori*-infected individuals.

The recommended treatment of *Helicobacter pylori*-associated diseases today includes a combination of two or more antibiotics with an inhibitor of acid secretion. However, even with these treatments, some bacteria may persist, in a non-growing, tolerant form or possibly intracellularly (5, 7, 10). This may lead to eradication failure, which is defined as the persistence of *H. pylori* at least 1 month after the end of antimicrobial therapy (9). Moreover, development of resistance of *H. pylori* strains to one or more of the antibiotics commonly used has been demonstrated previously (28). Thus, a need for new therapeutic agents that are effective against extracellular and potentially intracellular forms of *H. pylori* exists. We recently showed that sulforaphane, an isothiocyanate abundant in the form of its glucosinolate precursor in broccoli and broccoli sprouts, is a potent bactericidal agent against both extra- and intracellular *H. pylori* in vitro (11). Substantial quantities of isothiocyanates (up to 100 mg daily) and even greater quantities of their glucosinolate precursors are widely consumed by humans (12, 13, 29). They may act locally within the gastrointestinal tract or may distribute systemically after conversion to their cognate isothiocyanates (12, 15, 34). In this study, we investigated the efficacy of sulforaphane in vivo against *H. pylori* by using a recently developed model which uses human gastric xenografts in nude mice (22).

Sulforaphane, i.e., [(–)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane] was kindly provided by J. W. Fahey (Johns Hopkins University School of Medicine, Baltimore, Md.). Stock solutions were prepared in acetonitrile, and further dilutions were made in sterile water. *H. pylori* strain 26695, which was previously adapted for use in the gastric xenograft model (23), was used for graft inoculation. The strain was grown on Columbia agar supplemented with 10% horse blood under microaerobic conditions as previously described (22). For this isolate, the MIC of sulforaphane was 4 µg/ml, as determined by using the agar dilution method (pH 7.4) as recommended by

the National Committee for Clinical Laboratory Standard (NCCLS) (30).

Xenografts exhibiting human mature gastric epithelium and acidic secretion were obtained in nude mice as previously described (22) and with the approval of the French National Consultative Ethical Committee. Bacterial inoculation was performed by using a catheter that was implanted in the xenograft lumen (22). Two weeks after inoculation, each graft was microsurgically opened. Mucus was sampled for qualitative culture onto blood agar, and three biopsies were taken from antrum-adjacent sites for quantitative culture and histology. The levels of colonization and the concentrations of intracellular bacteria were determined by quantitative culture as previously described (23). For histological studies, specimens were processed by standard methods and stained with hematoxylin-eosin to assess the intensity of gastritis and with a modified Giemsa stain for detection of *H. pylori* (22).

The grafted animals were then randomly divided into two groups of 11. In the first group, 0.5 ml of sterile water with 0.5% acetonitrile containing 7.5 µmol of sulforaphane (approximately 1.33 mg) was administered via catheter once a day for 5 days. The same solution without sulforaphane was administered in the same way for 5 days in the other 11 infected grafts (control group). The effect of sulforaphane on *H. pylori* eradication was evaluated 4 weeks after treatment per the previous recommendation for human subjects (9). At that time, the animals were euthanized and the grafts were removed and opened. Three biopsy specimens were then taken from adjacent sites in the antrum for histological examination and determination of the level of mucosal colonization as well as the concentration of intracellular bacteria concentrations as described above. Eradication was defined by the absence of *H. pylori* detection by both histology and culture methods. Personnel performing this evaluation were masked (blinded) with regard to the treatment status. The MIC of sulforaphane was determined for all recovered isolates obtained from each culture-positive graft.

Before treatment, all 22 inoculated grafts were equally infected by *H. pylori* 26695, with no significant differences in numbers of CFU between the grafts that were destined to receive sulforaphane and the controls ($P = 0.34$, Mann-Whit-

* Corresponding author. Mailing address: Laboratoire de Bactériologie, Hôpital Central, 29 Avenue du Maréchal de Lattre de Tassigny, 54035 Nancy Cedex, France. Phone: (33) 3 83 85 21 96. Fax: (33) 3 83 85 26 73. E-mail: a.lozniewski@chu-nancy.fr.

TABLE 1. Effect of sulforaphane against *H. pylori* 26695 in human gastric xenografts

Treatment	No. of grafts in which <i>H. pylori</i> was eradicated ^a	Bacterial concn (log ₁₀ CFU/g) in antral mucosa ^b		No. of grafts with intracellular bacteria ^c /total no. of infected grafts	
		Before treatment	After treatment	Before treatment	After treatment
Control ^d (n = 11)	0	6.92 (6.00–7.44)	5.53 (6.59–7.81)	11/11	11/11
Sulforaphane ^e (n = 11)	8	6.80 (6.04–7.57)	5.60 (3.16–8.03) ^f	11/11	2/3

^a Eradication was defined by the absence of *H. pylori* as assessed by culture and histological examination of the gastric mucosa.

^b Values are expressed as means (ranges).

^c The presence of intracellular bacteria was assessed by culture of the gastric mucosa after incubation for 2 h in the presence of gentamicin.

^d Sterile water with 0.5% acetonitrile, once daily for 5 days.

^e Sterile water with 0.5% acetonitrile containing sulforaphane (7.5 μmol), once daily for 5 days.

^f As determined in the three grafts from which *H. pylori* was not eradicated.

ney U test) (Table 1). No organism other than *H. pylori* was recovered from either mucus or mucosal samples. Viable intracellular bacteria were also isolated in all the grafts and represented 0.04 to 2.5% (mean, 0.9%) of all the viable bacteria detected in the gastric mucosa. At that time, rare limited erythematous areas associated with hemorrhagic points or ulcerations were observed at the surface of the antrum in all inoculated grafts. Histological examination of antral biopsies confirmed the presence of *H. pylori* and showed mild inflammation and mild or moderate activity associated with diffuse interstitial edema. During the administration of sulforaphane or placebo (diluent vehicle control) and throughout the post-therapy period, no adverse reaction or change in weight or behavior was observed in the mice. Moreover, we did not observe any macroscopic or histopathological changes in internal organs at the end of the experiments. One month after the end of treatment, eradication was observed in 8 of the 11 sulforaphane-treated grafts (Table 1). Neither macroscopic abnormalities nor antral gastritis were observed in these culture-negative grafts. For the control group, there was no significant difference between the mucosal bacterial concentrations found before administration of the diluent and those found 5 weeks later ($P = 0.10$, Mann-Whitney U test) (Table 1). In sulforaphane-treated grafts that were not eradicated and in control grafts, both macroscopic and histological features were similar to those observed before treatment. Intracellular bacteria were detected in all control grafts (1 to 2.7% of all the viable bacteria detected) and in two of the three sulforaphane-treated grafts that were not eradicated (0.04 and 0.3% of all the viable bacteria detected). For isolates obtained from control grafts, no change in the MIC of sulforaphane was observed, whereas a fourfold increase of the MIC (to 16 μg/ml) was observed for all the isolates obtained from the three sulforaphane-treated grafts from which *H. pylori* was not eradicated. Such organisms were not detected after plating of the initial inoculum onto sulforaphane-containing agar.

A wide variety of plant extracts, i.e., individual plant components such as phytochemicals and phytochemical mixtures, have been shown to be active in vitro against *H. pylori* (1, 3, 4, 6, 8, 17–21, 24–26, 32, 33, 35, 36). A few of these phytochemicals have been tested in vivo (2, 14, 16, 20, 24, 27, 31), but clearance of *H. pylori* was observed only in two cases: after administration of tea catechins to *H. pylori*-infected gerbils (24) and after administration of goshuyu-to, a medicinal preparation which contains various plant extracts, including ginseng, to

humans (16). It is therefore critical to subject all plant compounds that are active against *H. pylori* in vitro to in vivo studies to determine their effectiveness in whole-organism systems, since most plant-derived compounds have not proven efficacious in such testing. Thus, having recently shown that sulforaphane was active against *H. pylori* in vitro and that it prevented the formation of benzo[a]pyrene-induced stomach tumors in ICR mice following an estimated daily intake of 7.5 μmol for 5 weeks (11), we next evaluated its potency in eradicating *H. pylori*, using the same dose for 5 days, in human gastric xenografts that were implanted in nude mice. In the present work, we have shown that sulforaphane has a significant effect against *H. pylori* in vivo, since we observed an eradication rate of 73% for the treated group and noted no eradication in the control group. Previous studies have demonstrated that sulforaphane, which has a concentration-dependent bactericidal activity against *H. pylori*, may accumulate in *H. pylori*-infected cells and reach intracellular levels at least fivefold higher than the administered concentration, suggesting that a reason for its bactericidal activity against intracellular bacteria may be related to its accumulation in mammalian cells (11). Whether intracellular accumulation of sulforaphane occurs in vivo is not yet known, but this may be one reason that the effectiveness of sulforaphane was not influenced in our study by the presence of intracellular bacteria. A failure of complete eradication was observed in only 3 of the 11 sulforaphane-treated grafts (due to the nature of the experiment, it was impossible to know whether there was a transient effect on *H. pylori* colonization), and such failure was always associated with the presence of isolates for which the MIC of sulforaphane was fourfold higher than that during the initial inoculation of an isolate. This apparent reduction in susceptibility may explain, at least partially, the failure of sulforaphane to completely eradicate *H. pylori* in these three xenografts. Other reasons, such as the existence of subtherapeutic antibiotic concentrations or poor stability of the drug at the site of infection, cannot be excluded.

We showed that *H. pylori* can be eradicated from human gastric xenografts after short-term administration of sulforaphane at a dose (1.33 mg/day in each xenograft [volume, ~7 ml]; 0.19 mg/ml) that can be achieved in the human diet (100 mg/day [stomach volume, 0.5 to 1 liter]; 0.1 to 0.2 mg/liter) (12, 13, 29). Thus, the administration of sulforaphane that can be safely delivered in the diet, particularly from broccoli sprouts, could be beneficial for the treatment of *H. pylori*-associated

gastric diseases. A sulforaphane-enriched diet might also be of value for prophylaxis against *H. pylori* infection and should be further evaluated. Xenografts represent the only in vivo model that permits the study of the effect of a compound on *H. pylori* interacting with fully differentiated human gastric mucosa. Since this model does not completely mimic the microenvironment of the infected human stomach, which is subject to the action of food, digestive physiology, and the potential coexistence of other bacterial species, further studies in humans are necessary to confirm the in vivo activity of sulforaphane against *H. pylori*.

We thank Jed W. Fahey, Katherine K. Stephenson, and Paul Talalay (Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, Md.) for their gift of sulforaphane and for their expert editorial advice.

REFERENCES

- Al Somal, N., K. E. Coley, P. C. Molan, and B. M. Hancock. 1994. Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *J. R. Soc. Med.* **87**:9–12.
- Aydin, A., G. Ersöz, O. Tekesin, and E. Akçiçek. 2000. Garlic oil and *Helicobacter pylori* infection. *Am. J. Gastroenterol.* **95**:563–564.
- Bae, E. A., M. J. Han, N. I. Baek, and D. H. Kim. 2001. In vitro anti-*Helicobacter pylori* activity of panaxytriol isolated from ginseng. *Arch. Pharm. Res.* **24**:297–299.
- Bae, E. A., M. J. Han, N. J. Kim, and D. H. Kim. 1998. Anti-*Helicobacter pylori* activity of herbal medicines. *Biol. Pharm. Bull.* **21**:990–992.
- Björkholm, B., V. Zhukhovitsky, C. Löfman, K. Hultén, H. Enroth, M. Block, R. Rigo, P. Falk, and L. Engstrand. 2000. *Helicobacter pylori* entry into human gastric epithelial cells: a potential determinant of virulence, persistence, and treatment failures. *Helicobacter* **5**:148–154.
- Boyanova, L., and G. Neshev. 1999. Inhibitory effect of rose oil products on *H. pylori* growth in vitro: preliminary report. *J. Med. Microbiol.* **48**:705–706.
- Brencia, M. L., A. M. Fornara, M. M. Scaltrito, and F. Dubini. 2000. *Helicobacter pylori*: cultivability and antibiotic susceptibility of coccoid forms. *Int. J. Antimicrob. Agents* **13**:237–241.
- Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12**:564–582.
- Dunn, B. E., H. Cohen, and M. J. Blaser. 1997. *Helicobacter pylori*. *Clin. Microbiol. Rev.* **10**:720–741.
- Engstrand, L., D. Y. Graham, A. Scheynius, R. M. Genta, and F. El-Zaatari. 1997. Is the sanctuary where *Helicobacter pylori* avoids antibacterial treatment intracellular? *Am. J. Clin. Pathol.* **108**:504–509.
- Fahey, J. W., X. Haristoy, P. M. Dolan, T. W. Kensler, I. Scholtus, K. K. Stephenson, P. Talalay, and A. Lozniewski. 2002. Sulforaphane inhibits extracellular, intracellular and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. *Proc. Natl. Acad. Sci. USA* **99**:7610–7615.
- Fahey, J. W., A. T. Zalcmann, and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **56**:5–51.
- Fenwick, G. R., P. K. Heaney, and W. J. Mullin. 1983. Glucosinolates and their breakdown products in food and food plants. *Crit. Rev. Food Sci. Nutr.* **18**:123–201.
- Graham, D. Y., S. Y. Anderson, and T. Lang. 1999. Garlic or jalapeño peppers for treatment of *Helicobacter pylori* infection. *Am. J. Gastroenterol.* **94**:1200–1202.
- Halliwell, B., K. Zhao, and M. Whiteman. 2000. The gastrointestinal tract: a major site of antioxidant action? *Free Radical Res.* **33**:819–830.
- Higushi, K., T. Arakawa, K. Ando, Y. Fujiwara, T. Uchida, and T. Kuroki. 1999. Eradication of *Helicobacter pylori* with a Chinese herbal medicine without emergence of resistant colonies. *Am. J. Gastroenterol.* **94**:1419–1420.
- Ingólfssdóttir, K., M. A. Hjalmsdóttir, A. Sigurdsson, G. A. Gudjonsdóttir, A. Brynjólfssdóttir, and O. Steingrímsson. 1997. In vitro susceptibility of *Helicobacter pylori* to protolicheterinic acid from the lichen *Cetraria islandica*. *Antimicrob. Agents Chemother.* **41**:215–217.
- Jones, N. L., S. Shabib, and P. M. Sherman. 1997. Capsaicin as an inhibitor of the growth of the gastric pathogen *Helicobacter pylori*. *FEMS Microbiol. Lett.* **146**:223–227.
- Kadota, S., P. Basnet, E. Ishii, T. Tamura, and T. Namba. 1997. Antibacterial activity of trichorabdol A from *Rabdosia trichocarpa* against *Helicobacter pylori*. *Zentbl. Bakteriol.* **286**:63–67.
- Kataoka, M., K. Hirata, T. Kunikata, S. Ushio, K. Iwaki, K. Ohashi, M. Ikeda, and M. Kurimoto. 2001. Antibacterial action of tryptanthrin and kaempferol, isolated from the indigo plant (*Polygonum tinctorium* Lour.), against *Helicobacter pylori*-infected Mongolian gerbils. *J. Gastroenterol.* **36**:5–9.
- Kubo, J., J. R. Lee, and I. Kubo. 1999. Anti-*Helicobacter pylori* agents from the cashew apple. *J. Agric. Food Chem.* **47**:533–537.
- Lozniewski, A., F. Muhale, R. Hatier, A. Marais, M. C. Conroy, D. Edert, A. Le Faou, M. Weber, and A. Duprez. 1999. Human embryonic gastric xenografts in nude mice: a new model of *Helicobacter pylori* infection. *Infect. Immun.* **67**:1798–1805.
- Lozniewski, A., X. Haristoy, D. A. Rasko, R. Hatier, F. Plénat, D. E. Taylor, and K. Angioi-Duprez. 2003. Influence of Lewis antigen expression by *Helicobacter pylori* on bacterial internalization by gastric epithelial cells. *Infect. Immun.* **71**:2902–2906.
- Mabe, K., M. Yamada, I. Oguni, and T. Takahashi. 1999. In vitro and in vivo activities of tea catechins against *Helicobacter pylori*. *Antimicrob. Agents Chemother.* **43**:1788–1791.
- Mahady, G. B., H. Matsuura, and S. L. Pendland. 2001. Allixin, a phytoalexin from garlic, inhibits the growth of *Helicobacter pylori* in vitro. *Am. J. Gastroenterol.* **96**:3454–3455.
- Mahady, G. B., and S. L. Pendland. 2000. Resveratrol inhibits the growth of *Helicobacter pylori* in vitro. *Am. J. Gastroenterol.* **95**:1849.
- McNulty, C. A. M., M. P. Wilson, W. Havinga, B. Johnston, E. A. O'Gara, and D. J. Maslin. 2001. A pilot study to determine the effectiveness of garlic oil capsules in the treatment of dyspeptic patients with *Helicobacter pylori*. *Helicobacter* **3**:249–253.
- Mégraud, F. 2003. *Helicobacter pylori* resistance to antibiotics: prevalence, mechanisms, detection. What's new? *Can. J. Gastroenterol.* **17**(Suppl. B):49B–52B.
- Mullin, W. J., and H. R. Sahasrabudhe. 1978. An estimate of the average daily intake of glucosinolate. *Nutr. Rep. Int.* **18**:273–279.
- National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Nir, Y., I. Potasman, E. Stermer, M. Tabak, and I. Neeman. 2000. Controlled trial of the effect of cinnamon extract on *Helicobacter pylori*. *Helicobacter* **5**:94–97.
- O'Gara, E. A., D. J. Hill, and D. J. Maslin. 2000. Activities of garlic oil, powder, and their diallyl constituents against *Helicobacter pylori*. *Appl. Environ. Microbiol.* **66**:2269–2273.
- Ohta, R., N. Yamada, H. Kaneko, K. Ishikawa, H. Kukuda, T. Fujino, and A. Suzuki. 1999. In vitro inhibition of the growth of *Helicobacter pylori* by oil-macerated garlic constituents. *Antimicrob. Agents Chemother.* **43**:1811–1812.
- Shapiro, T. A., J. W. Fahey, K. L. Wade, K. K. Stephenson, and P. Talalay. 2001. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. *Cancer Epidemiol. Biomark. Prev.* **10**:501–508.
- Tabak, M., R. Armon, and I. Neeman. 1999. Cinnamon's extract inhibitory effect on *Helicobacter pylori*. *J. Ethnol. Pharmacol.* **67**:269–277.
- Tabak, M., R. Armon, I. Potasman, and I. Neeman. 1996. In vitro inhibition of *Helicobacter pylori* by extracts of thyme. *J. Appl. Bacteriol.* **80**:667–672.