Rapid Recurrence of *Helicobacter pylori* Infection in Peruvian Patients after Successful Eradication

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Helicobacter pylori **is associated with gastritis, peptic ulcer disease, and gastric cancer. Since gastric cancer is common in Peru, eradication of *H. pylori* may help to reduce the occurrence of gastric cancer. This study involved three randomized trials to determine the efficacy of four different triple-drug therapy regimens. The most successful regimen was furazolidone combined with bismuth subsalicylate and amoxicillin, which eradicated infection in 82% of patients. Patients successfully treated were followed every 2–3 months to determine the recurrence rate of *H. pylori* infection. Of 105 patients with *H. pylori* eradication documented by pathology and culture, 52% (55) returned for follow-up endoscopy, and in 73% (40) of these 55 the infection recurred during the 8-month follow-up period. Thirty-five patients from whom *H. pylori* was eradicated and who were tested for antibodies to *H. pylori* remained consistently seropositive. Rapid recurrence of *H. pylori* infection after successful eradication suggests that measures other than antimicrobial therapy are needed to fight *H. pylori* in developing countries.**

In developing countries, *Helicobacter pylori* infection is important in the genesis of a progressive gastric pathology that predisposes to gastric cancer [1, 2], chronic active gastritis [3–5], duodenal ulcer [5–7], gastric ulcer [8], and gastric lymphoma [9]. Pathological changes associated with *H. pylori* infection appear to be progressive. Long-term eradication of *H. pylori* from the gastric mucosa may halt the progressive pathological process [3] and possibly serve as a cancer-control measure.

H. pylori transmission is incompletely understood but is thought to mainly occur via the fecal-oral route or fecally contaminated water supplies [10, 11]. In developing countries such as Peru, *H. pylori* infection is more common [12], occurs earlier in life [13], and is associated with more severe pathological lesions [14] than in developed countries.

In developed countries, 15 days of oral treatment with bismuth, metronidazole, and either amoxicillin or tetracycline eradicates *H. pylori* infections in 80%–90% of persons [15]. Resistance to metronidazole or its analogues generally does not exceed 25% [16, 17]. In contrast, >50% of *H. pylori* strains found in developing countries may be resistant to metronidazole or its analogues [18, 19], resulting in failure to eradicate *H. pylori* infection or in an early relapse due to incomplete microbial killing. Therefore, eradication of *H. pylori* infection by metronidazole-including regimens may be much less successful than in developed countries.

In developed countries, where standards of sanitation are high, the annual *H. pylori* infection rate is <1% [20], and reinfection after eradication is <5% per year [21]. The poor sanitation and prevalent fecal contamination common in developing countries results in reexposure to *H. pylori* that may permit rapid *H. pylori* reinfection. In the present investigation we sought to test, in the setting of a developing country, the efficacy of various antimicrobial regimens to eradicate *H. pylori* infection and the frequency or rate of *H. pylori* recurrence after successful eradication. In developed countries, seropositive patients become seronegative after successful treatment and eradication of *H. pylori* infection [22, 23]. This study used both biopsy and serology to demonstrate that infection recurred after appropriate therapy and eradication of *H. pylori.*

Materials and Methods

Patients. The study was approved by the institutional review boards of the Arzobispo Loayza Hospital (Lima), The Johns Hopkins University School of Hygiene and Public Health

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(Baltimore), and the Universidad Peruana Cayetano Heredia (Lima). Endoscopy was performed on informed, consenting adult patients of both sexes, aged 16–65 years, who presented with dyspeptic symptoms to the ambulatory endoscopy clinic at the Hospital Arzobispo Loayza and the Comas Clinic (Lima) between January 1989 and February 1992. Patients whose gastric biopsy specimen from either the antrum or the body of the stomach was positive for *H. pylori,* detected either by Warthin-Starry silver stain or by culture, were then eligible for enrollment into one of the three treatment trials.

Initial examination. Patients provided their medical history and demographic information, underwent a physical examination, and then underwent pretreatment endoscopy. To prevent cross-contamination, endoscopes were carefully sterilized between uses by means of successive washes with antimicrobial solution, acid/alcohol, and sterile water, as previously described [24, 25]. During each endoscopy, 5 biopsy specimens were taken: 3 from the antrum and 2 from the body of the stomach. One biopsy specimen from the antrum was cultured for *H. pylori,* while the remaining specimens were each stained with hematoxylin/eosin and Warthin-Starry silver stains and examined histologically, as previously described [25]. All biopsy specimens were coded, and all cultures and pathological readings were performed without knowledge of treatment status.

Evaluation of eradication. Three separate treatment trials were performed over 3 years. Patients in the first two trials were recruited mainly from an indigent population that attended Loayza Hospital; patients in the third trial mainly were recruited from a lower-middle-class population at the Comas Clinic. Patients were excluded from the study if they had an active gastric or duodenal ulcer, a history of allergic reactions or intolerance to any of the treatment medications, or any serious concomitant illness; if they were pregnant or breast-feeding; or if they had ingested antimicrobial agents or bismuth preparations in the past 15 days.

Patients were randomly assigned to one of two study arms in each of three trials. Randomization was performed with a computer program that generated random numbers. Patients in the different trials received different triple-drug antimicrobial therapies (table 1). The first two trials were randomized with a 3:1 ratio of cases to controls. Trial I patients were given amoxicillin (500 mg t.i.d.), bismuth subsalicylate (500 mg t.i.d.), and tinidazole (500 mg t.i.d.) (61 patients) or the respective placebos (24 patients) for 2 weeks. Trial II patients received tetracycline (250 mg t.i.d.), bismuth subsalicylate (500 mg t.i.d.), and tinidazole (500 mg t.i.d.) (66 patients) or the respective placebos (25 patients) for 2 weeks, followed by bismuth subsalicylate (500 mg t.i.d.) plus tetracycline (250 mg t.i.d.) or the respective placebos for 2 additional weeks. Trial III patients received amoxycillin (500 mg t.i.d.), bismuth subsalicylate (500 mg t.i.d.), and furazolidone (100 mg t.i.d.) (51 patients), vs. amoxycillin, bismuth subsalicylate, and metronidazole (500 mg t.i.d.) (50 patients) for 2 weeks.

Patients were asked to return 3–4 weeks after completion of their therapy for follow-up endoscopy, defined as the posttreatment endoscopy. Eradication therapy for *H. pylori* was considered successful if the patient's gastric biopsy was negative by both histologic stain and culture [25]. The absence of *H. pylori* by two methods (culture and histology) is accepted as evidence of cure [26]. Posttreatment failure was defined as relapse.

The study design in the first two trials (I and II) was double-blinded. In the third trial, only the physicians and endoscopists were blinded to differences in therapy among patients.

Evaluation of recurrence. Those patients from whom *H. pylori* was successfully eradicated were evaluated by endoscopy to determine the frequency of *H. pylori* recurrence over an 8-month study period. Patients were scheduled for endoscopy 2–3 months after eradication (defined as the first follow-up endoscopy), and if negative for *H. pylori,* they were asked to return 2–3 months later for an additional evaluation (defined as the second follow-up endoscopy). Patients who tested positive for evidence of recurrence on the first endoscopy were discharged from the study. Patients who were positive for *H. pylori* at the first or second follow-up endoscopy were considered to have had a recurrence.

Serological status. Blood samples were collected on admission, after completion of treatment, and at the time of follow-up endoscopies. Serum IgG antibodies to *H. pylori* were tested for by ELISA with use of a crude antigen, as previously described; this test has a sensitivity of 84% and a specificity of 92% [27]. All patients' sera were run in the same ELISA plate to avoid interplate differences. Two and a half years later, a subset of the cohort was tested again for antibodies to *H. pylori* by ELISA.

Statistical analysis. The proportion of patients whose therapy for eradication of *H. pylori* was successful was calculated for treatment and placebo groups in each study (table 1). Proportions were compared with use of χ^2 tests. Patients lost to follow-up and patients who became reinfected were compared with regard to age, sex, and socioeconomic status. A Kaplan-Meier curve was estimated for patient reinfection times (figure 1). Individual reinfection times for interval-censored observations were approximated at the midpoints. Patients were right-censored at the time of the last visit if they were negative by endoscopy at that visit. The estimated median reinfection time was calculated on the basis of the Kaplan-Meier estimates, as described by Brookmeyer and Crowley [28].

Results

Eradication. Of the 276 eligible patients, 73% (201) successfully finished the triple-drug therapeutic regimens. The median age was 35 years (ranging from 16 to 62 years), and the male:female ratio was 0.69.

No cases of spontaneous *H. pylori* eradication were observed in the placebo arms of groups I and II. Triple-drug therapy

Table 1. Success of eradication of *H. pylori* from Peruvian patients in three trial groups receiving different triple-drug antimicrobial therapies.

Trial group	Treatment received (2-w duration)	No. enrolled in study	Endoscopy for evaluation of eradication: No. (%) *H. pylori*–negative/no. treated
I	Amoxicillin (500 mg t.i.d.) Bismuth subsalicylate (500 mg t.i.d.) Tinidazole (500 mg t.i.d.)	61	22/45 (49)
	Placebo	24	0/13
II	Tetracycline (500 mg t.i.d.) Bismuth subsalicylate (500 mg t.i.d.) Tinidazole (500 mg t.i.d.) For 2 additional weeks: Tetracycline (250 mg t.i.d.) Bismuth subsalicylate (500 mg t.i.d.)	66	32/54 (59)
	Placebo	24	0/16
III*	Amoxicillin (500 mg t.i.d.) Bismuth subsalicylate (500 mg t.i.d.) Metronizadole (500 mg t.i.d.)	51	19/34 (56)
	Amoxicillin (500 mg t.i.d.) Bismuth subsalicylate (500 mg t.i.d.) Furazolidone (100 mg t.i.d.)	50	32/39 (82)

* Furazolidone, as a component of the triple-drug antimicrobial therapy, was two times more likely to improve eradication rates than was metronizadole ($P = .015$). The difference between those enrolled and those for whom eradication was successful represents the no. of patients who did not return for evaluation.

regimens including tinidazole and metronidazole eradicated *H. pylori* from 49%–59% of the patients. When metronidazole was replaced by furazolidone in a triple-drug therapy regimen that also contained amoxicillin and bismuth subsalicylate (trial III), the frequency of eradication increased from 56% to 82% ($P = .015$, χ^2).

Recurrence. In 52% (105) of 201 patients, *H. pylori* was successfully eradicated with initial therapy (posttreatment success). Of the 105 patients with documented eradication, 52% (55/105) returned for at least one additional endoscopy in an 8-month period. Recurrence of *H. pylori* infection occurred in 73% (40) of the 55 individuals followed during the 8-month study period (table 2). Frequencies of reinfection varied widely but not significantly ($P = .55$, χ^2) between the three different study trials (table 2). Trial II patients presented the highest proportion of recurrent cases among all study groups. The estimated median time of recurrence was 1.90 months (95% CI, 1.45–2.90 months; figure 1).

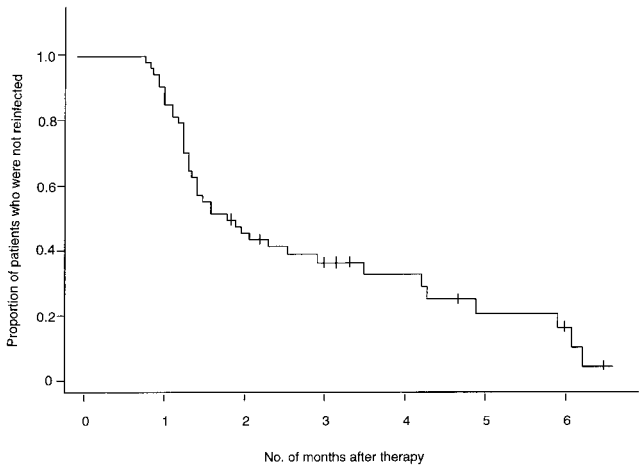

Figure 1. Time to recurrence of infection with *Helicobacter pylori* in 55 Peruvian patients after successful antimicrobial therapy (Kaplan-Meier curve). The vertical bars on the curve indicate the dropout times of patients who had not yet had a recurrence (i.e., right-censored patients).

Forty-eight percent (50) of the 105 *H. pylori*–negative patients did not return after eradication therapy, and 24 percent (13) of the 55 patients who returned for the first follow-up endoscopy did not return for the second (table 2). There was no significant difference between patients lost to follow-up and patients who had a recurrence with regard to age ($P = .53$), sex ($P = .63$), or socioeconomic status, as estimated by type of housing ($P = .27$), water source ($P = .09$), and sewage ($P = .60$). Nonetheless, even if all those who did not return would have remained negative, at the very least 38% (40/105) would have had a recurring episode of *H. pylori.*

Serology. Of the 105 patients from whom *H. pylori* was eradicated, 35 had sera samples still available for testing. Sera were available from 34 and 17 patients at 4 months and 2 years after eradication, respectively. All 35 patients were seropositive on admission (OD: 1.22 ± 0.25), and none seroconverted to negative over time.

Discussion

In developed countries, eradication of *H. pylori* infection by antimicrobial therapy is highly successful and long-lasting [29].

Table 2. Recurrence of *H. pylori* in Peruvian patients after successful eradication.

Trial group	Mean time (mo) to 1st follow-up endoscopy post-eradication (*n*)	Evaluation at 1st follow-up endoscopy*	Mean time (mo) to 2nd follow-up endoscopy (*n*)	Evaluation at 2nd follow-up endoscopy*	Evaluation after all follow-up endoscopies*
I	2.88 (14)	9/14 (64)	2.47 (3)	2/3 (67)	11/14 (79)
II	2.23 (17)	14/17 (82)	3.00 (3)	2/3 (67)	16/17 (94)
III[†]	2.12 (24)	13/24 (54)	NA	NA	NA
Total	2.32 (43)	36/55 (65)	2.73 (6)	4/6 (67)	40/55 (73)[‡]

NOTE. NA = not applicable.
* No. (%) of *H. pylori*–positive recurrences/no. of patients tested.
[†] This group includes patients from a pooling of both arms of the study.
[‡] Total of evaluations at 1st and 2nd follow-up endoscopies.

In contrast, *H. pylori* recurrence occurs frequently and rapidly in Peru. Our data provide further evidence that in less developed countries people are constantly exposed to *H. pylori* [10, 11] and that even recent infection provides little protection from recurrence.

The data presented also show that triple-antimicrobial regimens including metronidazole are less effective in Peru than in developed countries. Approximately 50% of the *H. pylori* strains in Peru are resistant to metronidazole [19], a condition highly correlated with triple-drug failure in developed countries [30]. Poorly regulated pharmacy practices and common overprescribing of antimicrobial drugs in less developed countries [18] also contribute to emerging resistance to *H. pylori.* Furazolidone may be useful against metronidazole-resistant strains, as shown by its high efficacy in comparison with the other regimens used in the present study.

The failure of Peruvian patients to seroconvert to negative may result from (1) a failure to completely eradicate *H. pylori* infection or (2) rapid reinfection after eradication. In developed countries, eradication of *H. pylori* infection in patients usually is associated with a steady decline in antibody titer and eventual seroconversion to negative over a 10-month period [23]. Eradication in Peruvian patients was not associated with either a decline in titer or seroconversion to negative.

Evidence supporting the *H. pylori* rapid-reinfection hypothesis includes the following: (1) therapies that eradicate *H. pylori* in patients were standard, as were the culture and silver stain methods used to detect *H. pylori;* (2) with use of the above therapy and methods, patients in developed countries in whom *H. pylori* infection is eradicated do not become reinfected rapidly. Our results suggest that rapid reinfection is occurring in poor Peruvian subjects and possibly in other areas where fecal-oral contamination is common. However, our data leave this question unanswered. Genetic analysis and comparisons of pretreatment isolates from patients with recurrent infection would be useful to resolve this issue.

H. pylori has been convincingly associated with gastric cancer and a premalignant lesion, chronic atrophic gastritis [2, 3]. Because of the high rates of gastric cancer in Peru, *H. pylori* eradication theoretically could play an important role in cancer prevention. Unfortunately, our data show that *H. pylori* recurrence occurs so rapidly that brief alleviation of gastritis with short-term antimicrobial therapy will most likely not change the course of the disease nor play a role in cancer prevention in less developed countries. In such countries, a vaccine to prevent *H. pylori* infection and long-range improvements in hygiene and sanitation appear to be better options.

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