0002-9270/93/88116-1860$03.00/0
THE AMERICAN JOURNAL OF GASTROENTEROLOGY

Vol. 88, No. 11, 1993
Printed in U.S.A.

Clarithromycin as Monotherapy for Eradication of *Helicobacter pylori*: A Randomized, Double-blind Trial

Walter L. Peterson, M.D., David Y. Graham, M.D., Barry Marshall, M.D., Martin J. Blaser, M.D., Robert M. Genta, M.D., Peter D. Klein, Ph.D., Charles W. Stratton, M.D., James Drnec, Ph.D., Philippe Prokocimer, M.D., and Nancy Siepman, Ph.D.

Medical Service, Dallas VA Medical Center, and Department of Medicine, UT Southwestern Medical School, Dallas, Texas; Departments of Medicine and Microbiology, Baylor College of Medicine, Houston, Texas; Department of Medicine, University of Virginia Medical School, Charlottesville, Virginia; Department of Medicine, Vanderbilt University Medical School, Nashville, Tennessee; Departments of Pathology and Medicine, Baylor College of Medicine, Houston, Texas; Departments of Pediatrics and Medicine, Baylor College of Medicine, Houston, Texas; and Abbott Laboratories, Abbott Park, Illinois

Current regimens to eradicate *Helicobacter pylori* usually consist of metronidazole plus a bismuth compound, as well as a third agent such as tetracycline. Such regimens are not ideal because organisms may be metronidazole-resistant, side-effects occur, and compliance is often poor. This randomized, double-blind study was designed to assess the ability of clarithromycin, a new macrolide antimicrobial, as monotherapy to eradicate *H. pylori*. Thirty-seven healthy volunteers who were *H. pylori* positive by ^{13}C-urea breath test plus histology and/or culture completed 14 days of oral therapy with clarithromycin in one of three dosages. Eradication, defined as all three tests negative at 4–6 wk after the end of therapy, was achieved in 2/13 (15%) with clarithromycin 500 mg *bid*, 4/11 (36%) with 1000 mg *bid*, and 7/13 (54%) with 500 mg *qid*. Isolates of *H. pylori* were resistant to clarithromycin prior to therapy in 12% of subjects, and became resistant during therapy in 21% of subjects. Taste perversion, the most common side effect, resulted in one subject terminating therapy. *Conclusions:* Whereas clarithromycin is a promising antimicrobial in the eradication of *H. pylori*, it is not sufficient to be used as monotherapy.

INTRODUCTION

Helicobacter pylori is the most common cause of chronic superficial gastritis in humans (1). *H. pylori* gastritis is present in most patients with duodenal and gastric ulcers (2) and is strongly associated with the intestinal type of gastric adenocarcinoma (3–5). Because eradication of *H. pylori* might cure peptic ulcers and, in parts of the world where it remains an important cause of death, decrease mortality from gastric cancer, more attention has turned toward antimicrobial therapy. The requirements for such therapy include high degrees of effectiveness and safety, coupled with low cost and incidence of side effects. To date, therapy with single antimicrobial agents has been relatively ineffective. For example, bismuth alone eradicates *H. pylori* less than 20% of the time (6). Erythromycin alone is poorly effective (7), possibly because it is acid-labile. Metronidazole or the quinolones alone also are ineffective, primarily because of the rapid emergence of resistance (8, 9). It has been necessary to add one or more antimicrobial agents to metronidazole to prevent the emergence of resistance (9). Therapy currently accepted as the gold standard includes metronidazole plus a bismuth compound and tetracycline or amoxicillin. Such triple therapy is highly effective against organisms susceptible to metronidazole (10, 11), although side effects of the regimen tend to reduce compliance. Furthermore, triple therapy is largely ineffective against metronidazole-resistant organisms (10, 11), a situation present in many persons who previously have received nitroimidazoles for other reasons. Therefore, efforts have intensified to find antimicrobial agents to replace metronidazole in combination regimens or, ideally, be effective as monotherapy against *H. pylori*. Clarithromycin is a new macrolide that is more acid-stable than erythromycin, has more predictable pharmacokinetics, and has excellent *in vitro* activity against *H. pylori* (12). A pilot study showed that 250 mg *qid* eradicated organisms in five (42%) of 12 subjects at 4- to 6-wk follow-up (13). The purpose of this randomized, double-blind, multi-center study was to evaluate several oral dosage regimens of clarithromycin as monotherapy to eradicate *H. pylori*.

METHODS

Experimental protocol

Subjects were healthy men and women between the ages of 18–65 yr; all were asymptomatic, had no history

Received Apr. 27, 1993; accepted Jul. 1, 1993.

of peptic ulcer disease, had a positive screening ^{13}C-urea breath test (UBT), and had taken no medication within the 2 wk prior to the study. After baseline gastroscopy with gastric mucosal biopsies for histologic examination and culture, subjects were randomly assigned to one of three dosages of clarithromycin for 14 days. They returned at 2–3 days and at 15–16 days (post-dosing) for repeat UBTs. If the post-dosing UBT was positive, a follow-up endoscopy with biopsy was performed without delay; if it was negative, follow-up endoscopy with biopsy (and UBT) was delayed for 4–6 wk to confirm that the organisms were truly eradicated and not merely suppressed. Eradication of *H. pylori* was defined by a negative UBT and absence of organisms by histology and culture 4–6 wk post-therapy. The study was approved by the Institutional Review Boards at each of the three participating centers, and each subject provided written informed consent.

Medications

Clarithromycin was provided as 500-mg tablets with matching placebo. To maintain the double-blind, all subjects received two tablets of clarithromycin or placebo four times daily. Subjects were randomly assigned to receive clarithromycin in dosages of either 500 mg *bid*, 1000 mg *bid*, or 500 mg *qid*. Patients who failed to take at least 70% of their medication were excluded from analysis of efficacy.

^{13}C-Urea breath test

The presence of urease in the stomach was determined by the ^{13}C-urea breath test as an indirect marker for the presence of *H. pylori* (14). After a baseline breath sample (20 ml) was collected, subjects consumed 5 ounces of Ensure pudding (Ross Laboratories, Columbus, OH) containing 250 calories, then drank 125 ml of water containing 125 mg ^{13}C-urea (Isotec Corp., Miamisburg, OH). Duplicate breath samples were collected at 20, 30, 40, and 50 min and were subsequently analyzed for excess $^{13}CO_2$ by gas isotope ratio mass spectrometry on a Roboprep G instrument (Europa Scientific, Crewe, UK). CO_2 production was calculated from body surface estimates based on the subject's weight and height (15, 16). Urease activity was expressed in μmol/min; test values in excess of 0.50 μmol/min were defined as positive for *H. pylori*.

Endoscopic biopsies

Four gastric biopsies were taken at each endoscopy: two biopsies from the greater curvature within 2–5 cm of the pylorus, and two from the lesser curvature within 1 cm of the angularis. One specimen from each location was placed in 10% buffered formalin for subsequent histologic examination, and the other was placed in cysteine Brucella broth with 20% glycerol and frozen at −70°C until cultured. Biopsy specimens, fixed in buffered formalin, were processed, oriented on edge, embedded in paraffin, and cut in sequential 5-μm sections. Virtually all specimens included surface epithelium and many also included muscularis mucosae. From each specimen, one slide (usually with eight to 12 sections) was stained with hematoxylin and eosin and one with the Warthin-Starry silver stain.

Histologic examination of biopsies

All biopsy specimens were reviewed by one pathologist (RMG) who was blinded to the treatment protocol of the patients. In each biopsy specimen, the number of neutrophils was graded on a semi-quantitative scale (devised prior to the start of the study) as follows: 0 = complete absence; 1 = rare, scattered neutrophils in the lamina propria; 2 = few scattered neutrophils also within the epithelium; 3 = moderate number of neutrophils; and 4 = large number of neutrophils, often with formation of pit abscesses. The number of mononuclear cells was graded on a similar scale, with the differences that 0 indicated the presence of a normal population of lymphocytes and plasma cells in the lamina propria, and a score of 4 indicated complete obliteration of the lamina propria by mononuclear cells, often also organized in lymphoid aggregates.

The intensity of *H. pylori* infection was evaluated by combining the observations of the hematoxylin and eosin-stained sections with those made on the Warthin-Starry-stained slides. This scale was graded as follows: 0 = no demonstrable organisms; 1 = less than 15 *H. pylori*-like organisms (HPLO) per slide; 2 = one to five HPLO per high power field (hpf); 3 = six to 20 HPLO/hpf; 4 = 21–100 HPLO/hpf; and 5 = >100 HPLO/hpf.

H. pylori culture and susceptibility testing

Biopsy specimens were delivered on dry ice to a central microbiology laboratory. Each specimen was ground in 0.5 ml saline and then inoculated to both blood and Skirrow's agar plates (BBL Microbiology Systems, Cockeysville, MD). Plates were incubated at 35°C in a microaerobic environment and examined every other day; plates showing no growth at 10 days were considered negative. Isolates were identified as *H. pylori* by Gram stain morphology, catalase, oxidase, and urease testing. For agar dilution susceptibility testing, strains were cultured on blood agar plates as required to yield good growth (usually 48–96 h). A heavy suspension of the growth in 1% peptone (Difco, Detroit, MI)–0.15 M saline broth was applied to freshly prepared agar plates for susceptibility testing with a Steers replicator. The medium consisted of Mueller-Hinton-supplemented agar plates (BBL) containing 2-fold dilutions of clarithromycin (0.06–8 μg/ml) plus 10% sheep blood, 10% fetal calf serum, and 4 μg/ml amphotericin B to prevent fungal overgrowth. Plates were incubated in a

microaerobic environment for 49–96 h and then scored for growth.

Statistical analyses

Pre-study UBT and histology results were compared among treatment groups using one-way analysis of variance. Fisher's exact test was used to compare eradication rates among treatment groups and the paired *t* test was used to compare the changes in histology results from pre-study to post-study. Ninety-five percent exact binomial confidence intervals for the eradication rates were calculated. Adverse events were categorized by the COSTART system (17). A subject reporting more than one adverse event for a particular COSTART term was counted only once. This incidence of adverse events, excluding concurrent conditions, was summarized by treatment group.

RESULTS

Subjects

Of the 43 subjects randomly assigned to the three treatment groups, six (three in each of the 2000 mg/day dosage groups) did not complete the study according to protocol. Three patients taking 1000 mg clarithromycin *bid* were withdrawn from the study prematurely: one because of noncompliance (<70% of study medication taken), another because of nausea, diarrhea, and taste perversion, and a third because of hepatitis C while participating in the study. Three patients taking 500 mg *qid* were also withdrawn from analysis: one was lost to follow-up, another took other antibiotics concurrently, and a third had no pretreatment cultures or histology performed. Pretreatment characteristics of the 37 evaluable subjects are shown in Table 1. There were no significant differences among the groups in prestudy

TABLE 1
Characteristics of Subjects Randomly Assigned to Each Dosage Regimen of Clarithromycin

	500 mg *bid* (n = 13)	1000 mg *bid* (n = 11)	500 mg *qid* (n = 13)
Age*	40.1 ± 3.63	37.9 ± 3.95	41.5 ± 3.22
Gender			
Men	9	5	5
Women	4	6	8
Race			
Asian	1	2	0
Black	6	3	4
White	4	3	8
Other	2	3	1
No. of smokers	2	2	3
^{13}C-UBT (μmol/min)*	2.19 ± 0.56	1.93 ± 0.44	2.19 ± 0.59
Density of *H. pylori* (0–5)*	4.3 ± 0.31	4.5 ± 0.28	3.3 ± 0.44†
PMN grade (0–4)*	2.8 ± 0.28	2.9 ± 0.37	2.8 ± 0.30
Monocyte grade (0–4)*	3.4 ± 0.18	3.2 ± 0.30	3.2 ± 0.23

* Mean ± SEM.
† $p < 0.05$ compared to other groups.

breath test results or grades of inflammatory cell infiltrate. Subjects randomized to the 500 mg *qid* group had a significantly lower density of *H. pylori* colonization by histology ($p < 0.05$); the mean score was skewed since this group included one culture-positive subject in whom *H. pylori* was not detected (grade 0) on either histologic specimen. This subject's mucosal PMN grade was 4.

Compliance with the medication regimens was excellent. Only two subjects were excluded from analysis because they did not take at least 70% of their medication. Of the 37 evaluable subjects, 35 (95%) took at least 50 of the possible 56 doses of medication.

Resistance of H. pylori to clarithromycin prior to therapy

H. pylori was isolated from 83 (99%) of 84 biopsy specimens obtained pretreatment from 42 subjects. One subject did not have pretreatment cultures and was excluded. Susceptibility testing was completed in 81/83 isolates, with 74 (91.3%) having minimal inhibitory concentration (MIC) values ≤2μg/ml, indicating susceptibilty to clarithromycin. Seven isolates from five different subjects (12% of the 42 subjects with pretreatment cultures) had MIC values ≥8, indicating resistance. Interestingly, the pair of isolates differed in their MICs by ≥four-fold in seven subjects, suggesting that they were infected by different strains at the two biopsy locations. Of the five subjects whose organisms were initially resistant, four completed the study according to protocol and three of them failed therapy.

Effect of clarithromycin on H. pylori infection

Eradication of *H. pylori*, defined as negative results from the UBT, culture, and histology 4–6 wk after cessation of therapy, occurred in 13 of 37 subjects. The eradication rates for subjects receiving 500 mg *bid*, 1000 mg *bid*, and 500 mg *qid* were 2/13, 15% (95% CI, 2–45%); 4/11, 36% (CI, 11–69%); and 7/13, 54% (CI, 25–81%), respectively ($p = 0.12$). *H. pylori* were isolated post-treatment from 20 of the 33 evaluable patients whose organisms were initially susceptible to clarithromycin. In seven (21%) of these 33, *H. pylori* had become resistant to the antimicrobial agent. Thus, development of resistance to clarithromycin accounted for seven (29%) of the 24 treatment failures. There were no significant differences in the frequency of development of resistance among the three treatment groups.

Twenty-four subjects failed therapy (*i.e.*, culture and/or histology was ultimately found to be positive). The UBT was negative in 21 (88%) of these 24 subjects, 2–3 days after beginning therapy, in 12 (50%) immediately post-dosing, and in three (12%) subjects 4–6 wk after cessation of therapy. There were no false-positive UBTs, either immediately post-dosing, or when done 4–6 wk after therapy. With only one exception, there was per-

fect correlation between the presence or absence of *H. pylori* by culture and by histology. One subject had a negative post-dosing UBT and underwent endoscopy 39 days later. Although the culture failed to grow, histology (and repeat UBT) were positive.

Eradication was not influenced by subject age, gender, ethnicity, smoking history, or prestudy density of *H. pylori*.

Effect of eradication of H. pylori on mucosal histopathology

Eradication of *H. pylori* was associated with a reduction in mean (±SE) PMN score from 3.0 ± 0.36 to 0.2 ± 0.10 ($p < 0.001$) and mononuclear cell score from 3.4 ± 0.24 to 2.2 ± 0.20 ($p = 0.002$). In subjects without eradication, neither the PMN nor mononuclear cell scores changed significantly, 2.7 ± 0.19 to 2.5 ± 0.19, and 3.2 ± 0.16 to 2.8 ± 0.17, respectively.

Side effects

Adverse events occurred in 37 (86%) of 43 subjects who received one or more dose of study medication. The events were generally mild or moderate in severity, and most frequently involved the digestive system (*e.g.*, nausea) or special senses (*e.g.*, bitter or metallic taste). Gastrointestinal events were reported by 2/13 (15%), 7/14 (50%), and 8/16 (50%), and taste perversion was reported by 4/13 (31%), 9/14 (64%), and 12/16 (75%) of the subjects who received 500 mg *bid*, 1000 mg *bid*, or 500 mg *qid*, respectively. Medication was discontinued prematurely in one subject because of side effects, and in another because of the discovery of an unrelated case of hepatitis C infection.

DISCUSSION

We have shown that clarithromycin, a more acid-stable macrolide, meets several requirements for effective eradication of *H. pylori*. First, preexisting resistance of *H. pylori* to clarithromycin was present in only 12% of the subjects, although it is possible that widespread use of this antimicrobial agent for other indications may result in a higher frequency of resistance in the future. Second, compliance with clarithromycin was excellent. Although most patients taking higher doses of clarithromycin reported taste perversion, only one subject discontinued the drug prematurely for this reason. Third, clarithromcyin at a dose of 500 mg *qid* eradicated *H. pylori* in 54% of the subjects, a substantially higher eradication rate than previously reported for single antimicrobial therapy.

The major causes of treatment failure were the development of resistance during therapy and inability of clarithromycin to eradicate susceptible organisms, accounting for 29% and 58% of failures, respectively, when all dosages were combined. Although most subjects whose organisms were initially resistant to clarithromycin failed therapy, they accounted for only 13% of treatment failures. Thus, selecting out such patients prior to therapy would have little effect on overall eradication rate.

Despite the relatively high rate of eradication of *H. pylori*, compared with other antimicrobial agents used as monotherapy, clarithromycin cannot be recommended for use in this manner. Results are not as good as those reported for triple therapy, and the development of resistance to clarithromycin in about one-fifth of isolates initially susceptible to the agent poses a concern. Therefore, it is likely that clarithromycin will need to be given with other agents to achieve maximum efficacy. At the least, clarithromycin may be a good alternative to metronidazole in triple-therapy regimens, especially for treatment of patients with metronidazole-resistant organisms. Another approach would be to combine clarithromycin with omeprazole, which, according to early reports, may result in eradication of up to 80% of the infections (18). Studies are underway evaluating such combination regimens.

Results of this study also provide some insight into the accuracy of diagnostic tests to detect *H. pylori* after antimicrobial therapy. Using either culture or histologic positivity as evidence that *H. pylori* was present, both tests had positive predictive values of 100%, while negative predictive values were 93% and 100%, respectively. The UBT also had a positive predictive value of 100%, but lower negative predictive values. This was especially true during the study and immediately after dosing, suggesting that the organism was suppressed but not eradicated. It is for this reason that the UBT should not be performed until at least 4–6 wk after cessation of therapy. A positive UBT 4–6 wk after completion of therapy is evidence of treatment failure, and follow-up endoscopy is unnecessary. If the UBT 4–6 wk after therapy is negative, a decision to verify eradication by endoscopic biopsy would depend upon the clinical situation. Alternatively, repeat UBT in several weeks might be useful. If endoscopy is undertaken, our results suggest that culture does not increase sensitivity. Since culture is, in most laboratories, more difficult than tissue staining, the only current reason to attempt isolation of *H. pylori* is if information regarding antimicrobial susceptibility is desired.

ACKNOWLEDGMENTS

Supported by the Department of Veterans Affairs and a grant from Abbott Laboratories. We wish to thank Ginger Lew, PA, Cora Barnett, Mary Walker, and Hilda Ratner for their assistance in conducting this study.

Reprint requests and correspondence: Walter L. Peterson, M.D., Digestive Diseases (111B1), Dallas VA Medical Center, 4500 S. Lancaster, Dallas, TX 75216.

REFERENCES

1. Peterson WL. *Helicobacter pylori* and peptic ulcer disease. N Engl J Med 1991;324:1043–8.
2. Graham DY. *Campylobacter pylori* and peptic ulcer disease. Gastroenterology 1989;96(suppl):615–25.
3. Parsonnet J, Vandersteen D, Goates J, et al. *Helicobacter pylori* infection in intestinal- and diffuse-type gastric adenocarcinomas. J Natl Cancer Inst 1991;83:640–3.
4. Parsonnet J, Friedman GD, Vandersteen DP, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–31.
5. Nomura A, Stemmermann GN, Chyou P, et al. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991;325:1132–6.
6. Chiba N, Rao BV, Rademaker JW, et al. Meta-analysis of the efficacy of antibiotic therapy in eradicating *H. pylori*. Am J Gastroenterol 1992;87:1716–27.
7. McNulty CAM, Gearty JC, Crump B, et al. *Campylobacter pyloridis* and associated gastritis: Investigator-blind, placebo-controlled trial of bismuth salicylate and erythromycin ethylsuccinate. Br Med J 1986;293:645–9.
8. Glupczynski CS, Labbe M, Burette A, et al. Treatment failure of ofloxacin in *Campylobacter pyloridis* infection. Lancet 1987;1:1096.
9. Goodwin CS, Marshall BJ, Blincow ED, et al. Prevention of nitroimidazole resistance in *Campylobacter pylori* by coadministration of colloidal bismuth subcitrate: Clinical and in vitro studies. J Clin Pathol 1988;41:207–10.
10. Logan RPH, Gummett PA, Misiewicz JJ, et al. One-week eradication regimen for *Helicobacter pylori*. Lancet 1991;338: 1249–52.
11. Bell GD, Powell K, Burridge SM, et al. Experience with "triple" anti-*Helicobacter pylori* eradication therapy: Side effects and the importance of testing the pre-treatment bacterial isolate for metronidazole resistance. Aliment Pharmacol Ther 1992;6:427–35.
12. Clarithromycin and azithromycin: Better erythromycins? Drug Ther Bull 1991;29:101–2.
13. Graham DY, Opekun AR, Klein PD. Clarithromycin for the eradication of *Helicobacter pylori*. J Clin Gastroenterol 1993;16:292–4.
14. Graham DY, Klein PD, Evans DG, et al. Simple noninvasive method to test efficacy of drugs in the eradication of *Helicobacter pylori* infection: The example of combined bismuth subsalicylate and nitrofurantoin. Am J Gastroenterol 1991;86:1158–62.
15. Shreeve WW, Cerasi E, Luft R. Metabolism of [2^{-14}C] pyruvate in normal, acromegalic, and HGH-treated human sunjects. Acta Endocrinol 1970;65:155–69.
16. Haycock GB, Schwartz CJ, Wisotsky DH. Geometric method for measuring body surface area: A height-weight formula validated in infants, children, and adults. J Pediatr 1978;93:62–6.
17. "COSTART" coding symbols for thesaurus of adverse reaction terms (3rd ed). Rockville, MD: Department of Health and Human Services, Food and Drug Administration, 1990.
18. Logan RPH, Gummett PA, Hegarty BT, et al. Clarithromycin and omeprazole for *Helicobacter pylori*. Lancet 1992;340:239.