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# Prolonged Effect of Omeprazole on the $^{14}$C-Urea Breath Test

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***Objectives:*** **We investigated omeprazole's effect on $^{14}$C-urea breath testing. We also determined the duration of omeprazole's effect on the breath test. Finally, we studied whether effects on breath testing were dose dependent.** ***Methods:*** **Fifty-seven employees and outpatients were screened for *Helicobacter* infection. Those positive for serology, CLO, or histology were asked to undergo baseline breath testing. Those with a positive breath test took omeprazole 20 mg/day for 14 days followed by repeat breath testing 1, 3, and 5 days after therapy. Subjects with persistently positive breath tests despite omeprazole 20 mg/day were asked to take omeprazole 20 mg *b.i.d.* for 14 days. Repeat breath tests were performed as above.** ***Results:*** **Thirteen of 57 had HP infection. Ten of 13 underwent a baseline breath test. Eight of 10 with baseline breath tests experienced a significant decrease in expired $^{14}CO_2$ after omeprazole 20 mg/day. Five of 13 with active HP infection developed a negative breath test after omeprazole. All subjects had a positive breath test within 5 days of stopping omeprazole 20 mg/day. Five of eight with persistently positive breath tests despite omeprazole 20 mg/day took omeprazole 40 mg/day. Four of five developed a significant decrease in $^{14}CO_2$ excretion after omeprazole. All subjects had a positive breath test within 5 days of stopping omeprazole 40 mg/day.** ***Conclusions:*** **Recent treatment with omeprazole 20 mg/day led to false-negative breath tests in 38.5%. This effect appeared to be dose dependent and lasted up to 5 days after cessation of omeprazole.**

## INTRODUCTION

*Helicobacter pylori* (HP) has emerged as an important factor in the pathogenesis of peptic ulcer disease and gastric carcinoma (1, 2). A number of diagnostic modalities are available to establish the presence of HP infection. Methods that rely upon the direct demonstration of the organism include histology and culture. Indirect methods identify HP's characteristic urease activity (CLO or urea breath testing) or demonstrate an antibody response to the organism (serological testing) (3).

Breath testing provides a means of documenting the presence of HP before or after specific therapy. Urea breath tests identify HP by virtue of its urease activity. $^{14}$C- or $^{13}$C-labeled urea is swallowed by the patient. If urease is present, urea is cleaved to form $HCO_3^-$ and $NH_4^+$. Ammonium is sequestered in gastric acid, but $HCO_3^-$ diffuses into the blood stream and is expired from the lungs in the form of labeled $CO_2$. Breath tests are noninvasive, reproducible, relatively inexpensive, and easy to perform. They have been found to be both sensitive and specific in the diagnosis of HP infection (3, 4). In the United States, neither the $^{13}$C-urea nor $^{14}$C-urea breath test is currently approved for clinical use by the FDA. However, experts agree that breath testing in some form will soon become an important and widely utilized tool for the diagnosis of HP (4).

A number of factors can negatively affect the accuracy of the urea breath test, including the recent use of agents with anti-HP activity (4). Omeprazole is a proton pump inhibitor that is commonly used for the treatment of various acid-peptic disorders. Omeprazole reportedly has anti-HP properties, probably related to its potent acid-suppressive effects, as well as via possible effects on the bacterial $H^+/K^+$ ATPases (5). Because of this anti-HP effect, investigators have found that omeprazole therapy taken at the time of breath testing may cause false-negative results. This observation has been based upon the experience of a few investigators with a small number of patients and has not been well documented in the medical literature (6, 7). The optimal timing of breath testing after the discontinuation of these agents has yet to be established. This issue has clinical importance with regard to the optimal performance of the breath test. In addition, this issue has practical relevance, inasmuch as patients taking omeprazole with ongoing dyspeptic symptoms often are unwilling to withhold their medication.

In the current study, we investigated the effect of omeprazole on the accuracy of the $^{14}$C-urea breath test. In addition, we performed serial studies after the discontinuation of omeprazole to determine the duration of the drug's effect on the breath test. Finally, we questioned whether the effect of omeprazole on the breath test was dose dependent.

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## MATERIALS AND METHODS

### *Study population*

Fifty-seven employees and patients (25 females and 32 males) were recruited from the outpatient clinics of the University of Michigan Medical Center. All subjects were more than 18 yr old and gave written informed consent. Women of child-bearing age were not eligible for this study because of the small doses of radiation associated with $^{14}$C-urea breath testing. None of the subjects enrolled were taking medications known to affect the $^{14}$C-urea breath test adversely (antibiotics, Pepto Bismol, carafate) or had a history of previous gastric surgery. All subjects were in good general health, and a physical examination before enrollment was normal. This protocol was approved by the Institutional Review Board of the University of Michigan Medical Center.

### *Effect of omeprazole on the sensitivity of the $^{14}$C-urea breath test*

Those individuals interested in enrolling in the study underwent a serology test for HP (Flex-sure, Smith, Kline Diagnostics, San Jose, CA) or, if clinically indicated, an upper endoscopy with gastric mucosal biopsy for CLO testing (Tri-Med Specialties, Charlottesville, VA) and/or histopathological examination. Those without evidence of HP infection were excluded from further analysis. Those found to have positive HP serology, CLO test, or histology were asked to undergo a baseline $^{14}$C-urea breath test. Most subjects enrolled were not taking omeprazole at the start of the study. Those taking omeprazole when the study began were asked to withhold their medication for at least 7 days before the baseline breath test. Individuals who had negative baseline breath tests were excluded from further study. Those who had positive baseline breath tests were asked to take omeprazole 20 mg/day for 14 days. Patients then underwent repeat breath testing 1, 3, and 5 days after the cessation of omeprazole therapy. Once a patient developed a positive breath test, no further breath tests were performed for this part of the protocol. The data obtained from these studies were used to calculate the rate of false-negative breath tests in patients recently taking omeprazole. In addition, the length of time during which the breath test remained falsely negative was determined.

A number of individuals with positive baseline breath tests remained positive after a 14-day course of omeprazole 20 mg/day. To address whether a higher dose of omeprazole might lead to the development of a false-negative breath test, these subjects were asked to take a 14-day course of omeprazole at a dose of 40 mg/day (20 mg taken twice daily). Repeat breath tests with the same study end points described above were then performed.

### *$^{14}$C-urea breath test*

For the $^{14}$C-urea breath test, we used the protocol developed by Tri-Med Specialties. Subjects fasted for at least 6 h prior to the study. On the day of the test, a baseline breath sample was obtained by instructing subjects to blow slowly through tubing fitted with a one-way valve into a hyamine-containing solution. A pH-sensitive color change from blue to clear indicated the collection of an adequate breath sample. Subjects next ingested a capsule containing $^{14}$C-urea (1 $\mu$Ci). Repeat breath samples were collected 10 and 20 min after the ingestion of $^{14}$C-urea. Upon completion of the study, the breath samples were analyzed for the presence of $^{14}$C with a scintillation counter (Beckman LS-7800). A positive test was defined as $>$ 200 dpm, indeterminate test 100–200 dpm, and negative test $<$ 100 dpm. These criteria have recently been validated by Peura and colleagues. They found a cut-off of $>$ 200 dpm for a positive study to be 97% sensitive and 95% specific (8). Using these criteria, all positive studies were found to have $>$ 200 dpm at the 10-min sample.

### *Statistical analysis*

The false-negative rate (FNR) was calculated with the formula: FNR = FN/TP + FN, where TP = true positives and FP = false positives. Disintegrations per minute were expressed as means ± SEM. Mean dpm values were compared by Student's *t* test for paired observations.

## RESULTS

### *Effect of omeprazole on the sensitivity of the $^{14}$C-urea breath test*

Of the 57 subjects screened, 13 (22.8%) had active HP infection, the diagnoses being based on gastric mucosal histology, CLO testing, or serological testing, followed by confirmatory $^{14}$C-urea breath testing. Of the 13 subjects enrolled, five were female and eight were male (mean age 47.3 yr). Ten of 13 subjects underwent a baseline $^{14}$C-urea breath test. Three subjects with positive histology for HP chose to omit the baseline breath test and proceed directly to therapy with omeprazole. Eight of 10 subjects with baseline breath tests experienced a significant decrease in expired $^{14}CO_2$ (1900 ± 214 dpm at baseline, 536 ± 161 dpm after omeprazole 20 mg/day, $p < 0.01$). Breath test data from all subjects enrolled are presented in Table 1. Five of 13 subjects with active HP infection had a negative breath test ($<$ 200 dpm) after 14 days of therapy with completion of omeprazole 20 mg/day. This yielded a false-negative rate of 38.5%. Three of the five subjects who had a negative breath test 1 day after omeprazole reverted to a positive breath test within 3 days of completing therapy. All five subjects negative 1 day after omeprazole had a positive breath test 5 days after cessation of the drug.

Five of eight subjects with a persistently positive breath test after omeprazole 20 mg/day agreed to undergo a 14-day course of omeprazole 40 mg/day (20 mg taken twice daily). Four of five subjects developed a significant decrease ($p <$ 0.02) in $^{14}CO_2$ excretion after omeprazole (Table 2). One of five developed a negative breath test, and two others had

TABLE 1
*Results of baseline breath tests and serial breath tests performed after a 14-day course of omeprazole 20 mg/day**

| Subject | Baseline | Day 1 | Day 3 | Day 5 |
|---|---|---|---|---|
| 1 | 2405 | 77 | 90 | 1388 |
| 2 | (+) Histology | 8 | 118 | 509 |
| 3 | 3360 | 54 | 238 | |
| 4 | (+) Histology | 59 | 1115 | |
| 5 | 2380 | 73 | 1803 | |
| 6 | 1222 | 648 | | |
| 7 | 1630 | 726 | | |
| 8 | 1028 | 1639 | | |
| 9 | 1658 | 1754 | | |
| 10 | 1674 | 593 | | |
| 11 | 1607 | 270 | | |
| 12 | (+) Histology | 700 | | |
| 13 | 2036 | 370 | | |
| Mean DPMs | 1900 ± 214 | 536 ± 161 | | |

* Results are expressed in dpm. Omeprazole led to a marked decrease in $^{14}CO_2$ excretion in 8/10 patients with baseline breath test results ($p < 0.01$). Results of serial breath testing 1, 3, and 5 days after omeprazole are also presented. A positive test was defined as >200 dpm, indeterminate test 100–200 dpm, and negative test <100 dpm. All subjects had a positive breath test 5 days after cessation of omeprazole.

TABLE 2
*Results of breath testing (dpm) in five subjects retreated with omeprazole 40 mg/day × 14 days**

| Subject | Baseline | Day 1 | Day 3 | Day 5 |
|---|---|---|---|---|
| 6 | 1222 | 48 | 60 | 412 |
| 7 | 1630 | 111 | 1542 | |
| 8 | 1028 | 131 | 1490 | |
| 9 | 1658 | 1446 | | |
| 10 | 1674 | 567 | | |
| Mean DPMs | 1900 ± 214 | 461 ± 263 | | |

* Five subjects who had persistently positive breath tests despite taking omeprazole 20 mg/day × 14 days agreed to be retreated with omeprazole at a dose of 40 mg/day × 14 days. This table documents results of breath testing (dpm) after omeprazole 40 mg/day. A positive test was defined as >200 dpm, indeterminate test 100–200 dpm, and negative test <100 dpm. Four of five subjects experienced a significant decrease in $^{14}CO_2$ excretion after this dosage of omeprazole ($p < 0.02$). One of five developed a negative breath test after the higher dosage of omeprazole. All five subjects had a positive breath test 5 days after stopping omeprazole.

indeterminate results after omeprazole 40 mg/day. Once again, all subjects had a positive breath test within 5 days of discontinuing the higher dosage of omeprazole.

## DISCUSSION

Urea breath tests are emerging as important tools in the diagnosis of HP (4). Versions of the urea breath test that use $^{14}$C and $^{13}$C are currently undergoing clinical trials in the United States in the hopes of achieving FDA approval. Once FDA approved, these tests will be widely available for use by clinicians. Thus, the establishment of optimal conditions for performing the test will be very important.

Omeprazole, a benzimidazole proton pump inhibitor, has been shown to have anti-HP properties and has been incorporated into a number of treatment regimens for this organism (9, 10). Proton pump inhibitors can inhibit the growth of HP *in vitro* (11). These agents may also directly inhibit HP urease activity (12). McGowan *et al.* (5) recently reported that omeprazole inhibited the growth of HP at low pH levels by a urease-independent mechanism. This effect was also observed with strains of bacteria other than HP. They speculated that HP might exert its toxic effects via binding of bacterial ATPases. The possibility that proton pump inhibitors exert anti-HP effects through mechanisms independent of their ability to raise gastric pH may explain why histamine 2-receptor antagonists in standard doses do not adversely alter the accuracy of the $^{14}$C-urea breath test (Barry Marshall, personal communication). The effect of high-dose histamine 2-receptor antagonist therapy on the $^{14}$C-urea breath test has not been established.

From a more clinical standpoint, Weil *et al.* (6) treated 24 HP-infected patients with omeprazole at doses of 20 or 40 mg/day for 28 days. They then assessed clearance (absence of HP at the end of treatment) and eradication (absence of HP >1 month after therapy), using the $^{14}$C-urea breath test. Three of 12 and 0/12, respectively, experienced clearance and eradication of HP after therapy with omeprazole at a dose of 20 mg/day. The numbers for clearance and eradication were 6/12 and 1/12, respectively, after omeprazole 40 mg/day (6). A more recent publication found that therapy with omeprazole alters results of $^{13}$C-urea breath testing. In this study, 4/29 (13.8%) developed a negative breath test ($^{13}CO_2$ excretion < 5 per mil) immediately after taking omeprazole 40 mg/day for 4 wk. All four of these patients had a positive breath test 2 wk after discontinuing omeprazole. The authors also used serial histological evaluations to demonstrate an improvement in antral gastritis and shift in density of *Helicobacter* organisms to the more proximal parts of the stomach after therapy with omeprazole (7).

These studies provide evidence that omeprazole adversely affects the accuracy of the $^{14}$C-urea breath test. However, they do not provide insight into the duration of this effect. Our study confirmed that recent treatment with omeprazole can lead to a false-negative breath test. Five of 13 (38.5%) subjects with active HP infection were found to have a negative breath test after 14 days of therapy with omeprazole 20 mg/day. In addition, if we assume that the level of expired $^{14}CO_2$ provides a semi-quantitative measure of HP urease activity, it is noteworthy that 8/10 subjects with a baseline breath test experienced a significant decrease in expired $^{14}CO_2$ immediately after omeprazole 20 mg/day, ($p < 0.05$). This observation suggests that omeprazole exerted a suppressive effect in the vast majority of subjects tested. Three of five subjects with a negative breath test 1 day after omeprazole tested positive within 3 days of discontinuing omeprazole. All five subjects negative 1 day after omeprazole had a positive breath test 5 days after stopping the drug. Five of eight subjects who had a persistently positive breath test after omeprazole 20 mg/day

agreed to undergo a 14-day course of omeprazole 40 mg/day (20 mg taken twice daily). One of five developed a negative breath test, and two others had indeterminate results after omeprazole 40 mg/day. Once again, all subjects had a positive breath test 5 days after cessation of omeprazole. $^{14}CO_2$ excretion after the higher dose of omeprazole was significantly lower than that observed after the course of omeprazole 20 mg/day, suggesting that omeprazole's effects were dose dependent.

This study has important practical implications for centers performing the $^{14}$C-urea breath test. Certainly, many patients taking omeprazole for ongoing dyspeptic symptoms prefer to withhold their medication only as long as absolutely necessary. Our data suggest that omeprazole should be withheld for a minimum of 5 days before the study. The concurrent use of an antibiotic or bismuth with omeprazole would necessitate a longer waiting period (>28 days) before $^{14}$C-urea breath testing. The newly approved proton pump inhibitor, lansoprazole, probably will also effect the accuracy of the $^{14}$C-urea breath test. However, whether the magnitude or timing of this effect will be the same as that observed with omeprazole remains to be established. Intuitively, there is no reason why these data could not also be applied to the $^{13}$C-urea breath test, although a study to confirm our results would be of interest.

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