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Studies Regarding the Mechanism of False Negative Urea Breath Tests With Proton Pump Inhibitors

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OBJECTIVE: The mechanism of false negative urea breath tests (UBTs) results among proton pump inhibitor (PPI) users is unknown. We studied the time course of PPI-associated negative UBT, the relation to *Helicobacter pylori* density, and whether gastric acidification would prevent false negative UBT results.

METHOD: In the UBT experiment, *H. pylori*–infected volunteers received omeprazole 20 mg *b.i.d.* for 13.5 days. UBTs with citric acid were done before, after 6.5 days of PPI, and 1, 2, 4, 7, and 14 days after therapy. In the culture and histology experiment, after a wash-out of >5 months, nine of the original subjects were rechallenged with omeprazole for 6.5 days. Antral and corpus biopsies for histology and culture were done before and 1 day after PPI administration.

RESULTS: Thirty subjects (mean age 42 yr) were enrolled. UBTs were significantly reduced on day 6.5 ($p = 0.031$); 10 subjects (33%) developed transient negative UBTs. The UBT recovered in all but one subject by the fourth day post-PPI and in all subjects by day 14. In the culture and histology experiment, upon PPI rechallenge, three of nine subjects (33%) had negative UBTs. *H. pylori* density, whether measured by culture or histology, decreased with PPI therapy; antral biopsies became histologically negative in five subjects and corpus biopsies in three subjects.

CONCLUSION: PPI-induced negative UBT results were related to the anti-*H. pylori* effect of the PPI. Acidification of the stomach did not prevent false negative UBT results. Three days is likely the minimum delay from stopping PPI until one should perform a test for active infection. A delay of 14 days is preferred. (Am J Gastroenterol 2003;98: 1005–1009. © 2003 by Am. Coll. of Gastroenterology)

INTRODUCTION

Proton pump inhibitors have a direct antibacterial effect on *Helicobacter pylori* and in addition have been reported to inhibit *H. pylori* urease activity (1–3). False negative urea breath test (UBT) results have been reported to occur in as many as 40% of individuals taking proton pump inhibitors (4–13). Although the mechanism(s) remains unclear, several, not mutually exclusive hypotheses have evolved to explain the phenomenon. One is related to the effect of the proton pump inhibitor on intragastric pH, which could make the intragastric environment unattractive for *H. pylori* and thus indirectly reduce the bacterial load. Alternatively, the pH could be increased sufficiently to close the postulated urea channel, UreI, and thus reduce urea's access to *H. pylori* urease (14–16).

Besides the effect on pH, the known direct anti–*H. pylori* antimicrobial effect of proton pump inhibitors could result in a direct reduction in bacterial load below the critical threshold of urea hydrolysis required for a positive test (8, 17–19). It has been postulated that the acidification of the stomach by co-administration of citric acid and urea would negate any pH effect of proton pump inhibitors and possibly prevent false negative UBT results (20). An alternate but not mutually exclusive explanation for a reduction in the proportion of false negative UBTs relates to the ability of citric acid to enhance *H. pylori* urease activity, possibly via enhanced delivery of urea to *H. pylori* urease (16, 17, 21). Thus, the citric acid–induced increase in the apparent urease activity could lower the threshold of bacteria needed to produce a positive UBT and reduce the proportion of tests with false negative results (17).

This series of experiments was designed to test these various hypotheses. For example, if the primary effect of the proton pump inhibitor was as an inhibitor of urease activity, one would expect false negative urease tests with no reduction in bacterial load, and the adverse effect would not be reversed by acidification of the stomach with citric acid. In contrast, one would expect citric acid to prevent false negative tests if the effect was primarily related to an increase in pH reducing access of urea to *H. pylori* urease. If the effect was primarily related to the antibacterial effect of the proton pump inhibitor, one would expect false negative urea breath tests to correlate with a reduction in bacterial load. We also asked how long one must delay urea breath testing to prevent false negative tests and whether the phenomenon was reproducible upon rechallenge.

MATERIALS AND METHODS

UBT Experiment

Healthy, *H. pylori*–infected volunteers underwent UBT testing using a commercially available UBT that contains 75 mg of ^{13}C-urea and 2 g of citric acid (BreathTek UBT for *H.*

pylori, Meretek, Nashville, TN) (21, 22). A positive UBT was defined as the enrichment of ^{13}C in the form of δ over baseline of 2.4 or greater. The volunteers then received omeprazole 20 mg *b.i.d.* for 13.5 days. The UBT was repeated on day 7 (after 6.5 days of proton pump inhibitor therapy), and day 15 (post-therapy day 1, or 24 h after the last dose of omeprazole). UBTs were also performed on post-therapy days 2, 4, 7, and 14. Breath testing was discontinued whenever two consecutive tests were positive.

Culture and Histology Experiment

In a separate experiment, after a wash-out period of >5 months, a subset of subjects who had participated in the first UBT experiment underwent urea breath testing and upper GI endoscopy. Then, omeprazole 20 mg *b.i.d.* was administered for 6.5 days, and the UBT was repeated. Endoscopy with biopsy was repeated on day 8 (approximately 24 h after ending the proton pump inhibitor therapy).

At each endoscopy, both antral and corpus biopsies were taken using jumbo forceps (Radial Jaw 3, Microvasive, Watertown, MA). Biopsies of the greater and lesser curvature of the mid-antrum (A2 and A5, respectively) and greater and lesser curvature of the mid-corpus (B4 and B6, respectively) were taken for histological grading. Each specimen was placed in a separate bottle of 10% buffered formalin, processed, and embedded on edge. Four-micron sections were stained with the El-Zimaity triple stain (23).

H. pylori density and polymorphonuclear cell and mononuclear cell infiltrations were scored using a semiquantitative analog scale ranging from 0 (none) to 5 (innumerable) (24).

Quantitative cultures were performed separately using one antral and one corpus biopsy. Briefly, each biopsy was removed from the transport medium (1 ml Brucella broth with 20% glycerol) and homogenized as described previously. The homogenized tissues were placed back into the 1 ml of transport medium and vortexed. Fifty microliters of each homogenized tissue specimen were removed and serially diluted 1:10 in sterile saline (10^0 to 10^{-6} dilution). Fifty-microliter aliquots of the diluted samples were removed from the dilution tubes and plated onto one nonselective (Brain Heart Infusion agar [BHIA; Difco Laboratories, Detroit, MI] containing 7% horse blood [Cocalico Biologicals, Reamstown, PA]) and one selective (BHIA containing antibiotics) horse blood agar plate. All plates were incubated at 37°C under 12% CO_2 for 7 days. The density of *H. pylori* in each biopsy was determined by multiplying the number of colonies on a plate by the dilution factor, then by the factor of 20. Results are expressed as the number of bacteria per biopsy specimen.

Effect of Omeprazole on H. pylori *In Vitro*

Thirty-four *H. pylori* isolates from volunteers completing the culture and histology experiments were tested by agar dilution to determine their susceptibility to omeprazole (AstraZeneca, Wayne, PA) *in vitro*. Briefly, isolates were recovered from frozen stocks and transferred twice on nonselective BHIA plates containing 7% horse blood. Fresh Mueller-Hinton agar (BBL, Becton Dickinson, Cockeysville, MD) plates containing omeprazole ranging in concentration from 0.125 μg/ml to 256 μg/ml were prepared. Each agar plate was inoculated with 3–5 μl of each adjusted inoculum [$(A)_{625} = 0.38-0.40$]. The plates were incubated at 37°C under CampyPak Plus (Becton Dickinson) conditions for 3 days. Minimum inhibitory concentration (MIC) values were defined as the lowest concentration of omeprazole that completely inhibited visible growth of the inoculum.

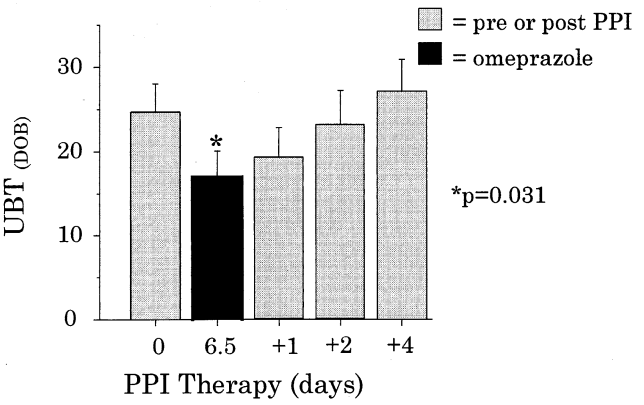

Figure 1. UBT results before, during, and after therapy with 20 mg of omeprazole *b.i.d.*. Omeprazole therapy was associated with a significant fall in mean UBT result ($p = 0.031$). DOB, delta over baseline.

Statistical Analyses

Data were analyzed using SigmaStat (SPSS, Chicago, IL) with two-tailed parametric or nonparametric tests, depending on whether the distribution of data was normal. Results are presented as mean ± SEM or, if the distribution was not normal, as medians. For analyses in which there were repeated measures, we used the one-way repeated measures analyses of variance. The pairwise comparison procedure was with the Tukey test.

The studies were approved by the Institutional Review Boards at Baylor College of Medicine and the VA Medical Center, Houston, TX. All patients provided written, informed consent before participation.

RESULTS

Thirty *H. pylori*–infected volunteers, 16 women and 14 men, mean age 42.5 yr (range 37–55 yr) were enrolled. Proton pump inhibitor therapy was associated with a decrease in the mean urease activity ($p = 0.031$) and did not return to the original baseline level until 2 days after ending proton pump inhibitor therapy (Fig. 1). Ten of the volunteers (33.3%) developed transiently negative UBT results, including nine subjects with negative UBTs at day 6.5 and nine subjects at day 14 (post-PPI day 1) (Fig. 2). Follow-up of the nine subjects with negative UBT test results at day 1 post-

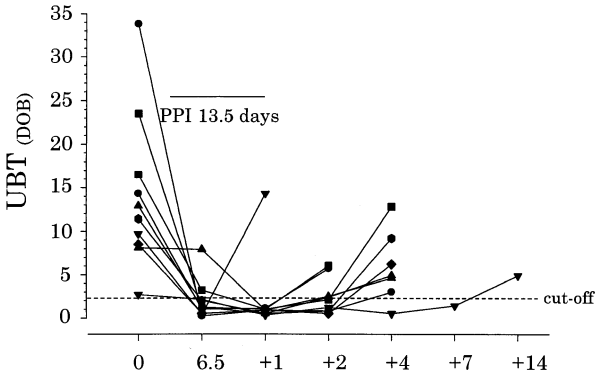

Figure 2. UBT results for the 10 subjects who developed transient false negative UBT results during therapy with omeprazole. All recovered by 14 days post-therapy. DOB, delta over baseline.

therapy showed that the tests remained negative in six subjects at day 2 post-therapy. All tests had reverted to positive by day 14 post-therapy (Fig. 2).

Culture and Histology Experiments

Nine subjects from the original study received a second omeprazole challenge of 20 mg *b.i.d.* for 6.5 days. These subjects included four of nine whose UBT became negative by day 6.5, one that had a positive UBT on day 6.5 but a negative UBT at day +1, and four whose UBT remained positive throughout the UBT experiment.

There was a direct correlation between the *H. pylori* density (*i.e.*, average number of *H. pylori* per biopsy in the antrum and corpus) and the UBT result ($R = 0.60$, $R^2 = 0.35$, $p = 0.009$) (Fig. 3).

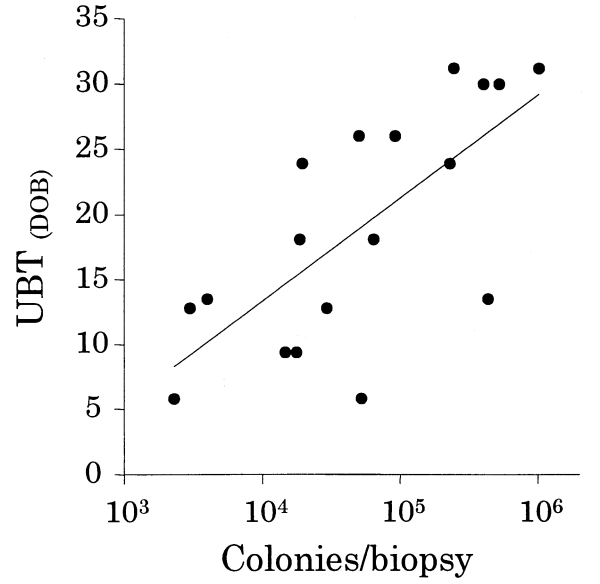

Figure 3. The relation between the UBT result and the quantitative culture results is shown. The results from both the antrum and corpus for each patient are plotted. The regression line is shown ($R = 0.6$, $R^2 = 0.31$, $p = 0.009$). DOB, delta over baseline.

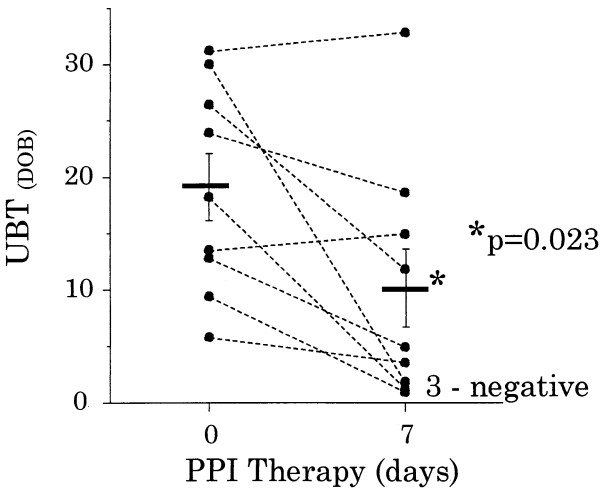

Figure 4. The effect of omeprazole 20 mg *b.i.d.* for 6.5 days is shown for the nine volunteers who were rechallenged. The mean UBT level fell, with three subjects (33%) having false negative UBT results. DOB, delta over baseline.

Omeprazole therapy resulted in a significant decrease in the mean UBT by day 7, with 33% of volunteers having negative UBT results (Fig. 4). Three of the four with prior false negative UBTs experienced a negative UBT, whereas none of those with positive UBTs throughout the first UBT experiment had negative UBTs in the second experiment. The fall in UBT value was accompanied by a fall in the density of *H. pylori* in both the antrum (median score from 3 to 0) ($p = 0.001$) and corpus (median score 2 to 1.5) ($p = 0.003$) as assessed by histology or quantitative culture (Fig. 5). This fall in *H. pylori* density resulted in many of the biopsies becoming negative for *H. pylori*. For example, before omeprazole therapy, 100% of the 36 gastric biopsies were histologically positive for *H.*

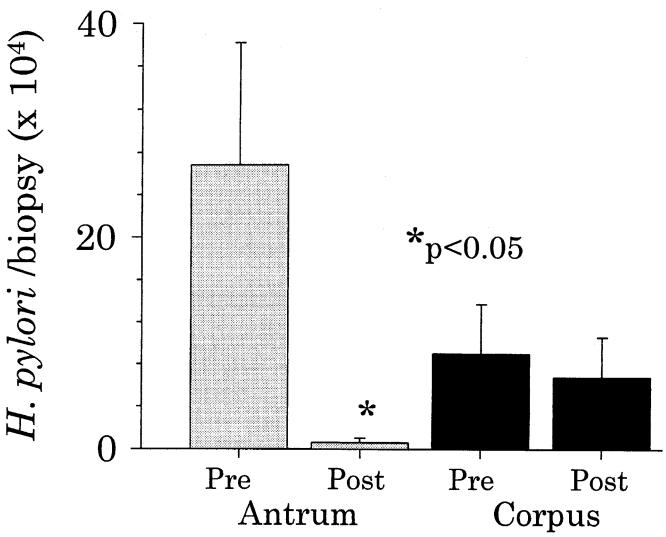

Figure 5. The results of quantitative culture for *H. pylori* before and after omeprazole therapy is shown separately for the antrum and the corpus. Pre-omeprazole, the *H. pylori*, density was higher in the corpus than in the antrum and fell more precipitously.

pylori. Post-therapy, only 19 (52.7%) showed *H. pylori*, and in many others the score was 1 (only one *H. pylori* per entire jumbo biopsy specimen). In 33% all biopsies in the antrum and corpus were negative for *H. pylori.*

Of the 34 isolates from nine subjects that were available for analysis of omeprazole susceptibility, 6/34 (18%) had an MIC of 16 μg/ml, 26/34 (76%) had an MIC of 32 μg/ml, and 2/34 (6%) had an MIC of 64 μg/ml. There was no correlation between the susceptibility pattern of the isolate and the UBT response.

DISCUSSION

The results of these experiments are consistent with the notion that the primary effect of proton pump inhibitor therapy is to reduce the density of *H. pylori* in the stomach (6–8, 19, 25–30). Prior studies were not designed to directly examine the relationship between *H. pylori* density and UBT result. The proportion of UBT with inability to detect *H. pylori* has varied, being 11% in the study by Logan *et al.*, 21% in our previous study, 33.6% in the study by Stolte and Bethke, and 33% here (6–8). Cultures were not negative but frequently had only one colony despite the use of jumbo forceps. Cultures were negative in 61% in the study by Kuipers *et al.* (29), but they used smaller biopsy specimens. These results are consistent with our previous study, in which we showed a relationship between the distribution of *H. pylori* in the stomach and the proportion of patients with negative ^{13}C-UBTs (8). For example, 40% of those with negative histology in either the antrum or corpus had false negative UBTs, compared with 11% of those with both antrum and corpus positive histologies (8).

Although there are data suggesting that high pH independent of proton pump inhibitor use (*e.g.*, with an H_2-receptor antagonist) may be associated with reduced urease activity as assessed by the UBT, the results have not been consistent, and the possible mechanisms are many (11, 31–36). Of interest, data from Parente *et al.* (37) suggested that proton pump inhibitors might differ in their ability to influence the UBT. In that study, pantoprazole use was not associated with false negative UBT results and, if confirmed, these results suggest that proton pump inhibitors may differ in their ability to influence *H. pylori* density in the stomach. The fact that pantoprazole is known to markedly influence gastric pH is consistent with pH not being a critical variable with regard to the ^{13}C-UBT. Nonetheless, the reported false negative results with H_2-receptor antagonists remain unexplained, and the mechanism is currently under investigation.

It is possible that one difference between the results reported relates to the form of the UBT. For example, it is possible that the mechanisms of false negative or false positive results with the ^{13}C- and the ^{14}C-UBTs may differ. Differences would be unlikely to be due to differences in the substrates but would more likely result from fundamental differences in the tests (22). The main difference between the two types of test is that the typical ^{14}C-UBT employs a minuscule amount of substrate, and the tiny amount of substrate makes substrate exhaustion, exit from the stomach before reacting with *H. pylori* urease, and/or insufficient amount for it to be distributed widely within the stomach potentially critical factors. This study directly addressed the hypothesis that acidification of the stomach with citric acid to "standardize" the intragastric pH would prevent false negative ^{13}C-UBT results; it did not (20). Because citric acid enhances urease activity *in vivo* (21), it is possible that the proportion with false negative UBT results was reduced compared with a test without citric acid, as citric acid could act to enhance urease activity among the population of surviving *H. pylori.* These results suggest that it may not be possible to construct a UBT that can overcome the marked reduction in *H. pylori* that occurs in some patients in association with proton pump inhibitor use (17). Although the small sample size of this study and the lack of data points between the intervals chosen made it impossible to define the exact interval needed to be completely confident that false negative tests had been excluded, the results are completely consistent with the results of large studies showing that 2 wk is sufficient (37) and that the minimum interval to discontinue proton pump inhibitors is at least 3 days before using the UBT to assess *H. pylori* status.

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REFERENCES

1. Nagata K, Takagi E, Tsuda M, et al. Inhibitory action of lansoprazole and its analogs against *Helicobacter pylori*: Inhibition of growth is not related to inhibition of urease. Antimicrob Agents Chemother 1995;39:567–70.
2. Mirshahi F, Fowler G, Patel A, Shaw G. Omeprazole may exert both a bacteriostatic and a bacteriocidal effect on the growth of *Helicobacter pylori* (NCTC 11637) in vitro by inhibiting bacterial urease activity. J Clin Pathol 1998;51: 220–4.
3. Stoschus B, Dominguez-Munoz JE, et al. Effect of omeprazole on *Helicobacter pylori* urease activity in vivo. Eur J Gastroenterol Hepatol 1996;8:811–3.
4. Weil J, Bell GD, Powell K, et al. Omeprazole and *Helicobacter pylori*: Temporary suppression rather than true eradication. Aliment Pharmacol Ther 1991;5:309–13.
5. Verdu EF, Frazer R, Armstrong D, Blum AL. Lansoprazole and omeprazole at therapeutic doses produce equivalent rises in gastric pH in *Helicobacter pylori* positive subjects. Gastroenterology 1995;A205:106 (abstract).

6. Stolte M, Bethke B. Elimination of *Helicobacter pylori* under treatment with omeprazole. Z Gastroenterol 1990;28:271–4.
7. Logan RP, Walker MM, Misiewicz JJ, et al. Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazole. Gut 1995;36:12–6.
8. Graham DY, Genta R, Evans DG, et al. *Helicobacter pylori* does not migrate from the antrum to the corpus in response to omeprazole. Am J Gastroenterol 1996;91:2120–4.
9. Bravo LE, Realpe JL, Campo C, et al. Effects of acid suppression and bismuth medications on the performance of diagnostic tests for *Helicobacter pylori* infection. Am J Gastroenterol 1999;94:2380–3.
10. Chey WD, Spybrook M, Carpenter S, et al. Prolonged effect of omeprazole on the 14C-urea breath test. Am J Gastroenterol 1996;91:89–92.
11. Chey WD, Woods M, Scheiman JM, et al. Lansoprazole and ranitidine affect the accuracy of the 14C-urea breath test by a pH-dependent mechanism. Am J Gastroenterol 1997;92:446–50.
12. Connor SJ, Seow F, Ngu MC, Katelaris PH. The effect of dosing with omeprazole on the accuracy of the 13C-urea breath test in *Helicobacter pylori*-infected subjects. Aliment Pharmacol Ther 1999;13:1287–93.
13. Laine L, Estrada R, Trujillo M, et al. Effect of proton-pump inhibitor therapy on diagnostic testing for *Helicobacter pylori*. Ann Intern Med 1998;129:547–50.
14. Scott DR, Weeks D, Hong C, et al. The role of internal urease in acid resistance of *Helicobacter pylori*. Gastroenterology 1998;114:58–70.
15. Weeks JL, Scott DR, Voland P, et al. The urease system of *Helicobacter pylori*. In: Hunt RH, Tytgat GNJ, eds. Helicobacter pylori: Basic mechanism to clinical cure 2000. Dordrecht: Kluwer Academic Publishers, 2000:15–24.
16. Shiotani A, Saeed A, Yamaoka Y, et al. Citric acid-enhanced *Helicobacter pylori* urease activity in vivo is unrelated to gastric emptying. Aliment Pharmacol Ther 2001;15:1763–7.
17. Graham DY. An alternate explanation of the effect of citric acid on proton pump inhibitor-associated false negative urea breath tests. Am J Gastroenterol 2001;96:3037–9.
18. Klein PD, Malaty HM, Martin RF, et al. Noninvasive detection of *Helicobacter pylori* infection in clinical practice: The 13C urea breath test. Am J Gastroenterol 1996;91:690–4.
19. Nakshabendi IM, Zhang QB, Mokhashi M, et al. Effect of omeprazole therapy on the survival of *Helicobacter pylori*, urease activity, and antral gastric histology in patients with duodenal ulcer. Helicobacter 1996;1:155–8.
20. Chey WD, Chathadi KV, Montague J, et al. Intragastric acidification reduces the occurrence of false-negative urea breath test results in patients taking a proton pump inhibitor. Am J Gastroenterol 2001;96:1028–32.
21. Graham DY, Runke D, Anderson SY, et al. Citric acid as the test meal for the 13C-urea breath test. Am J Gastroenterol 1999;94:1214–17.
22. Graham DY, Klein PD. Accurate diagnosis of *Helicobacter pylori*. 13C-urea breath test. Gastroenterol Clin North Am 2000;29:885–93.
23. El-Zimaity HM, Ota H, Scott S, et al. A new triple stain for *Helicobacter pylori* suitable for the autostainer: Carbol fuchsin/Alcian blue/hematoxylin-eosin. Arch Pathol Lab Med 1998;122:732–6.
24. El-Zimaity HM, Graham DY, Al-Assi MT, et al. Interobserver variation in the histopathological assessment of *Helicobacter pylori* gastritis. Hum Pathol 1996;27:35–41.
25. Suzuki M, Suzuki H, Kitahora T, et al. Proton pump inhibitor modifies inflammatory reaction in human gastric mucosa infected by *Helicobacter pylori*. Aliment Pharmacol Ther 2002; 16(suppl 2):229–34.
26. Schenk BE, Kuipers EJ, Nelis GF, et al. Effect of *Helicobacter pylori* eradication on chronic gastritis during omeprazole therapy. Gut 2000;46:615–21.
27. Fukuda Y. Suppression of *Helicobacter pylori* colonization with omeprazole. Scand J Gastroenterol Suppl 1996;214:54–5;discussion 57–60.
28. Uemura N, Okamoto S, Yamamoto S, et al. Changes in *Helicobacter pylori*-induced gastritis in the antrum and corpus during long-term acid-suppressive treatment in Japan. Aliment Pharmacol Ther 2000;14:1345–52.
29. Kuipers EJ, Uyterlinde AM, Pena AS, et al. Increase of *Helicobacter pylori*-associated corpus gastritis during acid suppressive therapy: Implications for long-term safety. Am J Gastroenterol 1995;90:1401–6.
30. Koop H, Stumpf M, Eissele R, et al. Antral *Helicobacter pylori*-like organisms in different states of gastric acid secretion. Digestion 1991;48:230–6.
31. Savarino V, Bisso G, Pivari M, et al. Effect of gastric acid suppression on 13C-urea breath test: Comparison of ranitidine with omeprazole. Aliment Pharmacol Ther 2000;14:291–7.
32. Connor SJ, Ngu MC, Katelaris PH. The impact of short-term ranitidine use on the precision of the 13C-urea breath test in subjects infected with *Helicobacter pylori*. Eur J Gastroenterol Hepatol 1999;11:1135–8.
33. Cutler AF, Elnaggar M, Brooks E, O'Mara K. Effect of standard and high dose ranitidine on [13C] urea breath test results. Am J Gastroenterol 1998;93:1297–9.
34. Stermer E, Tabak M, Potasman I, et al. Effect of ranitidine on the urea breath test: A controlled trial. J Clin Gastroenterol 1997;25:323–7.
35. Stermer E, Levy N, Tabak M, Neeman I. Lanzoprazole and ranitidine affect the accuracy of the 14C-urea breath test by a pH-dependent mechanism. Am J Gastroenterol 1997;92:1575–6 (letter).
36. Graham DY, Klein PD, Opekun AR, et al. In vivo susceptibility of *Campylobacter pylori*. Am J Gastroenterol 1989;84: 233–8.
37. Parente F, Sainaghi M, Sangaletti O, et al. Different effects of short-term omeprazole, lansoprazole or pantoprazole on the accuracy of the (13)C-urea breath test. Aliment Pharmacol Ther 2002;16:553–7.