Local Gastric and Serum Amoxicillin Concentrations after Different Oral Application Forms

M. P. COOREMAN, P. KRAUSGRILL, AND K. J. HENGELS*

*Departments of Gastroenterology and Medical Microbiology,
Heinrich-Heine-Universität, 4000 Düsseldorf, Germany*

The high recolonization rate after monotherapy of *Helicobacter pylori*-positive gastritis may be due to insufficient local drug concentrations. To investigate the role of local diffusion, we measured levels of amoxicillin, a drug with good in vitro activity against *H. pylori*, in the mucosa and serum. One gram of amoxicillin was given to healthy volunteers as a tablet ($n = 6$) or as water-dissolved, fizzing "Tab" ($n = 6$). Gastroscopy with biopsies from the antrum, corpus, and fundus was performed at 30, 60, and 90 min. Concentrations in the mucosa were measured after homogenization with the agar diffusion method using *Bacillus subtilis* as the biological indicator. Serum samples, taken basally and every 15 min, were analyzed by high-pressure liquid chromatography. Drug levels in the fundus and corpus remained far below those in the antrum for both application forms. The highest concentrations were reached after 30 min, with bactericidal levels in the antrum in two of six subjects who took the tablet form and five of six subjects who used Tabs. At 60 and 90 min, almost all values were below the MBC for 90% of the strains tested. The concentrations in serum, however, rose continuously, to reach a maximum after 75 or 90 min. These results show that incomplete elimination may be due to subbactericidal concentrations of antibiotics with high in vitro efficiency at the desired site of action in vivo and that local diffusion in the mucosa is essential for therapeutic effectiveness against *H. pylori*.

Worldwide and in all age groups, *Helicobacter pylori* is the major etiologic factor in chronic active antral gastritis (8, 9, 20, 28). There is also convincing evidence that *H. pylori* plays an important role in duodenal ulcer disease (2, 5, 12), especially since eradication of the bacterium is associated with a significantly lower recurrence rate compared with H2 receptor blocker therapy (6, 13, 19). Moreover, in up to 70% of patients with nonulcer dyspepsia, *H. pylori*-positive active antral gastritis can be diagnosed (18, 25), and although controversy about the clinical relevance of chronic gastritis remains, several investigators have found that these patients profit from elimination of *H. pylori* in terms of symptomatic improvement (14, 26). Bismuth salts, like colloidal bismuth subcitrate, and a variety of antibiotic drugs are able to eliminate the bacterium from the antral mucosa (15, 23), but recolonization within 1 month after therapy is the rule (24, 25). The reason for this incomplete elimination has not been sufficiently elucidated; it may be due either to reinfection from a bacterial reservoir in the upper gastrointestinal tract or to subbactericidal drug concentrations at the desired site of action during therapy. To investigate the latter possibility, we measured gastric mucosal concentrations of amoxicillin, a drug with excellent in vitro activity against *H. pylori*, given in the usual therapeutic dosage of 1 g in tablet form or in water-dissolved form. To compare the role of local diffusion of the drug with its systemic availability, we also determined the corresponding concentrations in serum.

MATERIALS AND METHODS

Eight healthy volunteers (seven females, one male; ages, 18 to 42 years) took part in the study after giving informed consent. The protocol was approved by the ethics committee of the University of Düsseldorf. Four volunteers participated twice, once with a tablet, and once with the water-dissolved form, defined as "Tabs," at an interval of more than 7 days.

Six subjects each received a tablet containing 1 g of amoxicillin (Amoxypen; Grünenthal GmbH, Stolberg, Germany) with a glass of water (ca. 50 ml) (group 1); in the second part of the study, six subjects each took the same amount of the drug dissolved in 50 ml of water, which gave an effervescent drink (Amoxypen Tabs; Grünenthal GmbH) (group 2). At 30, 60, and 90 min after ingestion of the drug, gastroscopy was performed and each time two biopsies were taken from the mucosa of the antrum, corpus, and fundus. After a biopsy had been taken, the pincers were rinsed thoroughly with sterile water. The combined weight of two biopsies from each region was measured without delay in preweighed Eppendorf caps, and the weights varied between 7.4 and 16.6 mg.

Microbiology. Amoxicillin concentrations were measured by the agar diffusion method with cavity inoculation of the ground biopsy with *Bacillus subtilis* (ATCC 6633) as the biological indicator and reference drug concentrations between 0.25 and 8.0 μg/ml (27). *B. subtilis* (2.4×10^7 bacteria per ml) was added to 80 ml of Müller-Hinton agar (Oxoid Ltd., Hampshire, Great Britain) and poured into a glass dish. After solidification of the agar, cavities were punched out and six of the caps were filled with the reference drug. The ground biopsies were placed into the remaining cavities. The glass dish was incubated at 37°C for 18 h. The diameters of the inhibition zones were then measured, and drug concentrations were determined on the basis of a calibration curve obtained with the reference amoxicillin concentrations. Results were expressed in micrograms per milliliter of biopsy tissue.
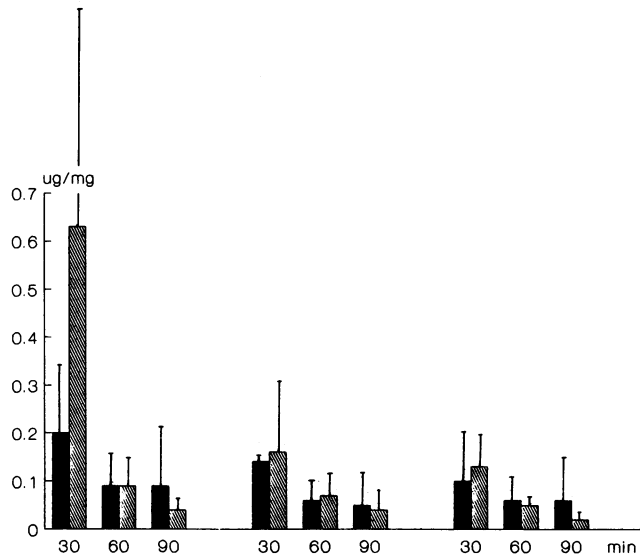
* Corresponding author.

FIG. 1. Amoxicillin levels in the antrum (left), corpus (center), and fundus (right) in six volunteers 30, 60, and 90 min after intake of amoxicillin in tablet (■) or dissolved-Tab (▨) form. Mean values and standard deviations are shown.

The MICs for 50 and 90% of *H. pylori* strains are 0.06 and 0.12 μg/ml, respectively, and the MBC_{90} is 0.25 μg/ml (11).

Measurement of serum amoxicillin concentrations. Venous blood samples were collected in 10-ml Vacutainers immediately before intake of the drug and every 15 min for 2 h after ingestion of the drug. Blood samples were immediately centrifuged, and the serum was stored at −20°C. Amoxicillin concentrations (in micrograms per milliliter) were measured by high-pressure liquid chromatography at the laboratories of Grünenthal GmbH in accordance with the methods described by Carlqvist and Westerlund (4).

Statistical analysis. For correlation of random tests (comparison of amoxicillin concentrations by localization, time interval, and drug application form), we used the Wilcoxon test. α values of <0.05 were considered statistically significant.

RESULTS

Amoxicillin concentrations in gastric mucosa. Irrespective of location and application form, the highest concentrations

of amoxicillin were measured in biopsy samples taken at 30 min (Fig. 1); drug levels were significantly higher in the antrum than in the corpus or fundus. After 60 and 90 min, all levels were below the MBC_{90} of 0.25 μg/mg, except for one subject in whom bactericidal levels in the antrum and fundus were observed 60 min after intake of the tablet form.

In two subjects in group 1 and five subjects in group 2, bactericidal levels in the antrum were found at 30 min (Table 1). Only after ingestion of the tablet form were bactericidal levels reached in the fundus in two subjects (at 30 min in subject 5 and at 60 min in subject 6). After intake of both the tablets and the Tabs, most amoxicillin levels in the corpus and fundus were far below the MBC_{90}. The differences between the concentrations in the antrum and fundus, however, did not reach statistical significance, maybe because of the relatively small number of volunteers and the fact that the range of concentrations was large. Mean values and standard deviations for both application forms are shown in Fig. 1.

Serum concentrations of amoxicillin. Serum levels of amoxicillin were very low at 15 min, after both Tabs and tablets. In six of the patients, no amoxicillin could be detected, and in only three patients were concentrations above the MBC_{90}. The levels increased constantly and reached a maximum after 75 or 90 min, with ranges from 2.48 to 13.02 (Tabs) and 3.69 to 15.15 (tablets) μg/ml after 75 min. Between 90 and 120 min, the concentrations fell continuously (Fig. 2).

DISCUSSION

Patients with *H. pylori*-associated conditions seem to benefit from elimination of the bacterium from the gastric mucosa, but early recolonization is a major problem of current therapeutic regimens (21, 25, 26). The recolonization rate after monotherapy with bismuth salts or amoxicillin is about 60 to 70% within 4 weeks (25). Superior results were reported after treatment with a combination of two or more antibiotic drugs, especially after triple therapy including, e.g., metronidazole, amoxicillin, and bismuth subcitrate (3). Except for treatment of duodenal ulcer disease that had been resistant to conventional therapy, the risks of possible toxicity, side effects, and development of bacterial drug resistance do not justify the widespread use of antibiotic combinations for relatively harmless conditions such as antral gastritis, especially since there controversy about the clinical relevance of gastritis in nonulcer dyspepsia remains (10, 14, 17, 26).

The reason for early recolonization after therapy has not

TABLE 1. Amoxicillin concentrations in the gastric mucosa of six volunteers 30 min after intake of 1 g of amoxicillin in tablet or dissolved Tab form

Drug form and biopsy site	Amoxicillin concn (μg/ml) in subject no.:						Range ($\bar{x}$ ± SD) of amoxicillin concn (μg/ml)
	1	2	3	4	5	6	
Tablets							
Antrum	0.12	0.04	0.28[a]	0.09	0.21	0.42[a]	0.04–0.42 (0.20 ± 0.14)
Corpus	0.08	0.02	0.26[a]	0.08	0.25[a]	0.16	0.02–0.26 (0.14 ± 0.09)
Fundus	0.09	0.01	0.19	0.04	0.29[a]	0.02	0.01–0.29 (0.10 ± 0.11)
Tabs							
Antrum	0.88[a]	0.12	0.41[a]	1.71[a]	0.37[a]	0.28[a]	0.12–1.71 (0.63 ± 0.58)
Corpus	0.43[a]	0.08	0.13	0.26[a]	0.04	0.03	0.03–0.43 (0.16 ± 0.15)
Fundus	0.19	0.24	0.11	0.05	0.12	0.06	0.05–0.24 (0.13 ± 0.07)

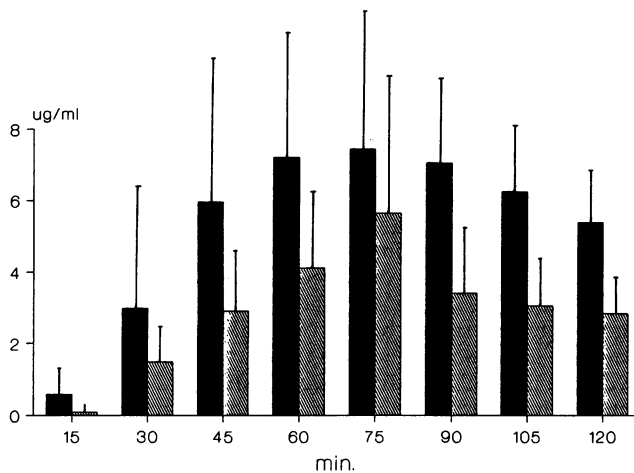[a] This value is greater than the MBC_{90}.

FIG. 2. Serum amoxicillin levels after 1 g in tablet form (▉) or as dissolved Tabs (▨). Mean values and standard deviations are shown.

been sufficiently elucidated. DNA sequence analysis has proved that in most cases recolonization is due to incomplete elimination and not reinfection (16). A bacterial reservoir in the upper gastrointestinal tract, where the drug concentration does not reach bactericidal levels, may play an important role in the development of antral recolonization.

Amoxicillin is a generally well-tolerated antibiotic drug with good in vitro activity against *H. pylori* and has therefore been used in many clinical trials aimed at eradication of the bacterium from the gastric mucosa (11, 22, 25). However, the results of these trials were disappointing because of rapid bacterial recolonization. The results obtained with amoxicillin given in combination with bismuth salts were only slightly better (25). The usual application form of the drug is a tablet, containing 750 or 1,000 mg of the active substance, given undissolved at a dosage of about 3 g daily. The tablet form, however, may cause inhomogeneous distribution of the drug in the mucosa. For this reason, we compared it with Tabs which were dissolved in water to form a fizzing drink before ingestion.

Our results show that 30 min after oral intake of 1 g of amoxicillin in tablet form, drug concentrations in the antral mucosa were subbactericidal in four of six subjects and were above the MBC_{90} in only two volunteers. However, *H. pylori* can also be isolated from the mucosa of the gastric corpus and fundus in patients with chronic antral gastritis (7). In these regions, drug levels were far below bactericidal concentrations and often hardly detectable. In the fundus and corpus, bactericidal concentrations were reached in two subjects and one subject, respectively. The results obtained with the same dosage of the soluble form (Tabs) of amoxicillin were significantly better, reaching bactericidal levels in the antral mucosa in five of six subjects 30 min after intake. Drug concentrations in the corpus and fundus were subbactericidal in all but two cases (corpus) and did not differ significantly from those measured with the tablet form.

Biopsy specimens may contain up to 80% cellular fluid. It might therefore be argued that the drug concentrations measured in this study were artificially low because of dilution of the drug with drug-free intracellular water. As β-lactam antibiotics do not penetrate well into the intracellular space, the measured concentrations should be multiplied by a maximum factor of 5 to correct for this effect. We

believe, however, that this dilution factor is of little practical relevance. Normal mucosal biopsies are about 1.5 to 2 mm in diameter, with a depth of 200 to 300 nm (2a), and the mucus layer covering the mucosa has a thickness of 50 to 450 μm (mean, 180 μm) (1). As a consequence, on average, ca. 50% of the volume of a gastric mucosal biopsy consists of mucus and the remaining 50% is mucosal tissue, reducing the dilution factor due to cellular fluid to less than 40%. Moreover, not all cells of a biopsy specimen are in lysis after grinding, reducing the dilution factor further.

Even with a dilution factor of 80%, the estimated concentrations are below the MBC_{90} or at the bactericidal level in most of the cases only at 30 min. While the actual dilution factor might be much less (<40%), this potential artifact would not considerably alter the results and would consequently have no impact on our conclusions.

In all of the subjects, the drug levels in the mucosa were highest 30 min after oral intake, independently of the application form and the location in the stomach. The lowest concentrations were measured at 90 min, and most of them were hardly detectable. Concentrations in serum, on the other hand, were very low or not detectable at 15 min and then rose continuously to reach a maximum after 75 min. Therefore, we assume that the amoxicillin concentrations we measured in the mucosa were the result of local diffusion; this also suggests that systemic distribution of amoxicillin is of little importance for its activity against *H. pylori*.

Accordingly, inhomogeneous local distribution and poor systemic availability at the site of desired action may contribute considerably to the unsatisfactory results of amoxicillin therapy with respect to long-term bacterial clearance. Little work has been done to investigate the importance of the galenic form for treatment of *H. pylori* gastritis. Our results suggest that we need more information on the local availability of antibiotics in the gastric mucosa and that we should look for galenic forms other than tablets to improve the results of anti-*H. pylori* treatment.

In conclusion, our data show that the dissolved form of amoxicillin is superior to the tablet form with regard to drug concentrations in the antrum. With the tablet form, subbactericidal levels in the antral mucosa may explain the persistence of *H. pylori* after amoxicillin monotherapy. With a dosage of 1 g, given as a tablet or in the dissolved form, concentrations remain far below the required bactericidal levels in the mucosa of the corpus and fundus. This makes a reservoir function of these parts of the stomach for *H. pylori* likely. Further studies with, e.g., higher dosages of the dissolved form with monitoring of concentrations in the mucosa are therefore needed to find a solid basis for future therapeutic trials aimed at eradication of *H. pylori*.

REFERENCES

1. **Allen, A., W. J. Cunliffe, J. P. Pearson, and C. W. Venables.** 1990. The adherent gastric mucus gel barrier in man and changes in peptic ulceration. J. Int. Med. **228**(Suppl.)**732**:83–90.
2. **Bayerdörffer, E., G. Kasper, T. Pirlet, A. Sommer, and R. Ottenjan.** 1987. Ofloxacin in der Therapie Campylobacter-pylori-positiver Ulcera duodeni. Dtsch. Med. Wochenschr. **112:** 1407–1411.
2a.**Borchard.** Personal communication.
3. **Börsch, G., U. Mai, and W. Opferkuch.** 1988. Oral triple therapy may effectively eradicate Campylobacter pylori in man: a pilot study. Gastroenterology **94:**44.
4. **Carlqvist, J., and D. Westerlund.** 1985. Automated determination of amoxycillin in biological fluids by column switching in ion-pair reversed-phase liquid chromatographic systems with post-column derivatisation. J. Chromatogr. **344:**285–296.
5. **Caselli, M., L. Trevisani, A. Aleotti, M. R. Bovolenta, and G.**

Stabellini. 1989. Gastric metaplasia in duodenal bulb and Campylobacter-like organisms in development of duodenal ulcer. Dig. Dis. Sci. 34:1374–1378.

6. Coghlan, J. G., H. Humphries, C. Dooley, C. Keane, D. Gilligan, D. McKenna, E. Sweeney, and C. O'Morrain. 1987. Campylobacter pylori and recurrence of duodenal ulcers—a 12-month follow-up study. Lancet ii:1109–1111.

7. Cohen, H., M. Gramisu, P. Fritzgibbons, M. Appleman, M. Skoglund, and J. E. Valenzuela. 1989. Campylobacter pylori: associations with antral and fundic mucosal histology and diagnosis by serology in patients with upper gastrointestinal symptoms. Am. J. Gastroenterol. 84:367–371.

8. Dooley, P. D., H. Cohen, P. L. Fritzgibbons, M. Bauer, M. D. Appleman, G. I. Perez-Perez, and M. J. Blazer. 1989. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. N. Engl. J. Med. 321:1562–1566.

9. Drumm, B., P. Sherman, E. Cutz, and M. Karmali. 1987. Association of Campylobacter pylori on the gastric mucosa with antral gastritis in children. N. Engl. J. Med. 316:1557–1561.

10. Elta, G. H., R. Murphy, E. M. Behler, J. L. Barnett, T. T. Nostrant, S. Kern, and H. Appleman. 1989. Campylobacter pylori in patients with dyspeptic symptoms and endoscopic evidence of erosions. Am. J. Gastroenterol. 84:643–646.

11. Goodwin, C. S., P. Blake, and E. Blincow. 1986. The minimum inhibitory and bactericidal concentrations of antibiotics and anti-ulcer agents against Campylobacter pyloridis. J. Antimicrob. Chemother. 17:309–314.

12. Graham, D. Y. 1989. Campylobacter pylori and peptic ulcer disease. Gastroenterology 96:615–625.

13. Humphreys, H., S. Bourke, C. Dooley, D. McKenna, B. Power, C. T. Keane, E. C. Sweeney, and C. O'Morrain. 1988. Effect of treatment on Campylobacter pylori in peptic disease: a randomised prospective trial. Gut 29:279–283.

14. Kang, J. Y., H. H. Tay, A. Wee, R. Guan, M. V. Math, and I. Yap. 1990. Effect of colloidal bismuth subcitrate on symptoms and gastric histology in non-ulcer-dyspepsia. A double blind placebo controlled study. Gut 31:476–480.

15. Lambert, J. R., K. L. Dunn, H. Turner, and M. G. Korman. 1986. Effect on histological gastritis following eradication of Campylobacter pyloridis. Gastroenterology 90:1509.

16. Langenberg, W., E. A. J. Rauws, A. Widjojokusumo, and G. N. J. Tytgat. 1986. Identification of Campylobacter pyloridis isolates by restriction endonuclease DNA analysis. J. Clin. Microbiol. 24:414–417.

17. Loffeld, R. J. L. F., H. V. J. P. Potters, E. Stobberingh, J. A. Flendrig, J. P. Van Spreeuwel, and J. W. Arends. 1989. Campy-lobacter associated gastritis in patients with non-ulcer dyspepsia: a double blind placebo controlled trial with colloidal bismuth subcitrate. Gut 30:1206–1212.

18. Loffeld, R. J. L. F., E. Stobberingh, J. A. Flendrig, J. P. Van Spreeuwel, and J. W. Arends. 1989. Diagnostic value of an immunoassay to detect anti-Campylobacter pylori antibodies in non-ulcer dyspesia. Lancet i:1182–1185.

19. Marshall, B. J., C. S. Goodwin, J. R. Warren, R. Murray, E. D. Blincow, S. J. Blackbourn, M. Phillips, T. E. Waters, and C. R. Sanderson. 1988. Prospective double-blind trial of duodenal ulcer relapse after eradication of Campylobacter pylori. Lancet ii:1437–1442.

20. Marshall, B. J., and R. H. Warren. 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet i:1311–1315.

21. Martin, D. F., E. Montgomery, A. S. Dobek, G. A. Patrissi, and D. A. Peura. 1989. Campylobacter pylori, NSAIDS, and smoking: risk factors for peptic ulcer disease. Am. J. Gastroenterol. 84:1268–1272.

22. McNulty, C. A. M., J. Dent, and R. Wise. 1985. Susceptibility of clinical isolates of Campylobacter pyloridis to 11 antimicrobial agents. Antimicrob. Agents Chemother. 28:837–838.

23. McNulty, C. A. M., J. C. Gearty, B. Crump, M. Davis, I. A. Donovan, V. Melikan, D. M. Lister, and R. Wise. 1986. Campylobacter pyloridis and associated gastritis: investigator blind placebo controlled trial of bismuth salicylate and erythromycin ethylsuccinate. Br. Med. J. 293:645–649.

24. Rauws, E. A. J. 1989. Therapeutic attempts at eradication of Campylobacter pylori. Eur. J. Gastroenterol. Hepatol. 1:34–41.

25. Rauws, E. A. J., W. Langenberg, H. J. Houthoff, H. C. Zanen, and G. N. J. Tytgat. 1988. Campylobacter pyloridis-associated chronic active antral gastritis. A prospective study of its prevalence and the effects of antibacterial and antiulcer treatment. Gastroenterology 94:33–40.

26. Rokkas, T., C. Pursey, E. Uzoechina, L. Dorrington, N. A. Simmons, M. I. Filipe, and G. E. Sladen. 1988. Non-ulcer dyspepsia and short term De-nol therapy: a placebo controlled trial with particular reference to the role of Campylobacter pylori. Gut 29:1386–1391.

27. Rosin, H., H. J. Hagedorn, M. Köhler, and L. Thomas. 1977. Agardiffusionsmethode; Rundtischgespräch über Methoden und Indikationen für Blutspiegelbestimmungen von Aminoglykosiden. Infection 5:3–8.

28. Stolte, M., S. Eidt, M. Ritter, and B. Bethke. 1989. Campylobacter pylori und Gastritis. Assoziation oder Induktion? Pathologe 10:21–26.