ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1999, p. 1387–1392 Vol. 43, No. 6
0066-4804/99/$04.00+0

Activities of β-Lactams and Macrolides against *Helicobacter pylori*

IBRAHIM J. HASSAN,[1]* ROGER M. STARK,[1] JOHN GREENMAN,[2] AND MICHAEL R. MILLAR[1]

Pathology and Microbiology Department, University of Bristol, Bristol BS2 8HW,[1] and Faculty of Applied Sciences, University of the West of England, Frenchay, Bristol BS16 1QY,[2] United Kingdom

Received 17 August 1998/Returned for modification 10 December 1998/Accepted 24 March 1999

A continuous-culture system (chemostat) was used to study the activities of β-lactam antimicrobial agents, clarithromycin, and 14-OH-clarithromycin against slowly growing *Helicobacter pylori* NCTC 11637. *H. pylori* was grown to steady state before exposure to these antimicrobial agents at ×8 the MIC. The bactericidal actions of combinations of amoxicillin and clarithromycin were also studied. Viable counts (numbers of CFU per milliliter) were determined at 2-h intervals for 12 h and at 20 h after the addition of antibiotics. The effects of pH changes (6.5 to 7.4) on the activities of amoxicillin, clarithromycin, and the combination of these against *H. pylori* NCTC 11637 were also studied. Viable counts following exposure to ampicillin, cefixime, ceftazidime, cefuroxime, cefotaxime, azlocillin, and piperacillin at 20 h showed bacteriostatic activity. Imipenem, meropenem, amoxicillin, clarithromycin, and 14-OH-clarithromycin reduced the viable counts by 3 $\log_{10}$ CFU/ml (≥99.9% killing). Imipenem was the most rapidly bactericidal against *H. pylori* NCTC 11637. Results of the pH experiments showed that amoxicillin was bactericidal at pHs 6.5 to 7.4. Clarithromycin was bactericidal at pH 7.0 to 7.4 but was bacteriostatic at pH 6.5. The combination of amoxicillin and clarithromycin was bactericidal at pHs 6.5 and 7.0. A batch culture (flask system) was also used to investigate 12 strains of *H. pylori* for their susceptibilities to β-lactams, clarithromycin, and/or 14-OH-clarithromycin in order to determine whether results from the chemostat model can be reproduced with batch cultures. Results of the chemostat time-kill kinetic study were reproducible in our batch culture flask system. The role of carbapenems in the eradication of *H. pylori* should be investigated.

Over the past 15 years *Helicobacter pylori* has emerged as an important human pathogen associated with gastroduodenal pathology (9, 10, 38). It is the principal cause of type B antral gastritis and contributes to the etiology of peptic ulcer disease. Eradication of the organism from the gut of patients reduces the relapse rate of peptic ulcer disease (35). It has also recently been epidemiologically linked to the development of gastric malignancies (7, 11, 43) and has been classified as a category 1 carcinogen by the World Health Organization (25, 36). Although *H. pylori* is susceptible to a wide range of antimicrobial agents in vitro (29), this does not translate into in vivo efficacy, and eradication requires the use of combinations of antimicrobial agents (5, 16, 23).

We report on a comparison of the activities of β-lactam antibiotics and macrolides against slowly growing *H. pylori* strains in continuous and batch cultures. The effect of pH on the activities of amoxicillin and clarithromycin alone and in combination against *H. pylori* NCTC 11637 in the chemostat was also studied. The investigations of bactericidal agents active against *H. pylori* in vitro may contribute to better strategies for eradication therapy. These may also help to determine if the in vivo success of clinical treatment regimens can be predicted by a chemostat model.

MATERIALS AND METHODS

Bacterial strains. *H. pylori* NCTC 11637 was used in all the chemostat experiments and, together with 11 other strains (NCTC 11916 and 10 clinical isolates) obtained at endoscopy from patients at the Bristol Royal Infirmary, Bristol, United Kingdom, between 1993 and 1996, was used for the batch culture kinetic study.

Media. The growth medium was a modified brucella broth (0495-17-3; Difco Laboratories, Detroit, Mich.) containing the following (per liter): Bacto Tryptone, 10 g; Bacto Peptamin, 10 g; Bacto Dextrose, 1 g; Bacto Yeast Extract, 2 g; NaCl, 5 g; and $NaHSO_3$, 0.1 g. This mixture was supplemented with 1% fetal calf serum (Gibco BRL, Uxbridge, United Kingdom).

MIC determination. The MICs of the antimicrobial agents used for the experiments were determined by a standard agar dilution method (24) with Columbia agar base (Oxoid CM 331; Unipath Ltd., Basingstoke, United Kingdom) supplemented with 5% horse blood. The plates were inoculated with a multipoint replicator (Mast, Liverpool, United Kingdom) with an inoculum of 10^4 CFU/spot. The plates were incubated at 37°C under microaerophilic conditions for 72 h before reading of the results. The MIC was defined as the lowest antibiotic concentration which prevented visible growth of bacteria. Repeat determinations were done by a broth macrodilution method as described previously (24). The interassay variation was no more than ±1 dilution step (except for clarithromycin at pH 6.5, for which a twofold dilution was observed). The results obtained by both methods were comparable to those reported previously (18).

Antimicrobial agents. The antimicrobial agents used in this study were ampicillin and amoxicillin (Beecham Laboratories, Betchworth, United Kingdom), imipenem (MSD Ltd., Hoddesdon, Herts, England), meropenem (ICI plc Macclesfield, Cheshire, England), piperacillin and cefixime (Lederle Laboratories, Gosport, Hants, England), azlocillin (Bayer Ltd., Berkshire, United Kingdom), ceftazidime and cefuroxime (Glaxo, Middlesex, England), cefotaxime (Roussel Laboratories Ltd., Uxbridge, England), and clarithromycin (Abbott Laboratories, Queenborough, United Kingdom). Each antimicrobial agent was prepared prior to use in accordance with the manufacturer's instructions, with a stock solution of known potency being produced.

Establishment of cultures in the chemostat. The chemostat used for the experiments was an LH 500 series direct-drive fermentor with control modules for temperature, pH, gas flow, and stir rate (Inceltech UK Ltd., Reading, Berks, United Kingdom). It consists of a culture vessel fitted with an overflow device to maintain a constant volume (15, 41). The method used in the present study for establishment of a stable continuous culture of slowly growing *H. pylori* was a modification of that described previously (32); namely, *H. pylori* was harvested from Columbia agar plates, suspended in 7 ml of sterile brucella broth that had been prewarmed to 37°C, and inoculated aseptically into 700 ml of modified brucella broth in the growth vessel of the chemostat. A microaerophilic gas mixture consisting of O_2, CO_2, and N_2 at 5:10:85 (in percent [vol/vol]), respectively, was filtered through a 0.2-μm-pore-size filter and was sparged at a rate of 300 ml/min through the chemostat vessel. The stir rate was maintained at 700 rpm, while the pot volume at this stir rate was 700 ml. Sterile growth medium from the medium reservoir was fed by a peristaltic pump into the culture vessel at a rate of 700 ml/24 h to give a dilution rate of 0.04 h^{-1}. The maximum specific growth rate (μ_{max}) of *H. pylori* NCTC 11637 under these conditions was 0.052 at pH 6.5, 0.132 at pH 7.0, 0.212 at pH 7.2, and 0.180 at pH 7.4. The incubation

* Corresponding author. Mailing address: Pathology and Microbiology Department, University of Bristol, BRI Level 8, Bristol BS2 8HW, United Kingdom. Phone: 44-117-9282514. Fax: 44-117-929 9162. E-mail: I.J.Hassan@bristol.ac.uk.

TABLE 1. MICs of antimicrobial agents for *H. pylori* strains

Strain	MIC (μg/ml)										
	Imipenem	Meropenem	Ampicillin	Amoxicillin	Clarithromycin	14-OH-clarithromycin	Cefixime	Cefuroxime	Cefotaxime	Piperacillin	Azlocillin	Ceftazidime
NCTC 11637	0.06	0.06	0.25	0.06	0.03	0.06	0.50	0.50	1.00	0.25	0.25	2.00
NCTC 11916	0.06	0.12	0.12	0.12	0.03	NT[a]	1.00	0.50	0.50	0.50	0.50	1.00
18868	0.12	0.25	0.25	0.25	0.12	NT	0.25	1.00	1.00	0.50	0.50	2.00
2985	0.06	0.25	0.12	0.12	0.06	NT	0.50	1.00	0.50	0.50	0.50	1.00
3571	0.12	0.24	0.50	0.25	0.24	NT	1.00	1.00	1.00	1.00	1.00	2.00
3266	0.12	0.25	1.00	0.50	0.50	NT	0.50	0.50	0.50	0.50	0.50	0.50
2245	0.06	0.12	0.25	0.12	0.06	NT	0.50	0.50	0.50	1.00	1.00	1.00
ABBA	0.50	2.00	2.00	2.00	2.00	NT	2.00	1.00	2.00	1.00	1.00	2.00
AMMI	1.00	1.00	4.00	2.00	2.00	NT	4.00	2.00	2.00	2.00	2.00	4.00
HAWA	0.06	0.12	0.25	0.25	4.00	NT	0.50	1.00	0.50	1.00	0.50	2.00
TIYO	4.00	2.00	4.00	2.00	0.03	NT	4.00	2.00	2.00	2.00	2.00	4.00
KAL	0.12	0.06	0.25	0.25	0.06	NT	0.50	0.50	1.00	0.50	0.50	1.00

[a] NT, not tested.

FIG. 1. Killing kinetics of antimicrobial agents (at 8× MIC) at pH 7.0 in chemostat cultures for *H. pylori* NCTC 11637. ■, imipenem; ☼, meropenem; ▼, Amoxicillin; ▲, clarithromycin; ◆, 14-OH-clarithromycin; ×, washout; →, time of antibiotic addition.

temperature was 37°C. The medium flow was started when the broth became visibly turbid and the chemostat culture was then allowed to stabilize over the next 5 to 6 days, permitting the establishment of a steady state between bacterial growth and bacterial washout, at which point the killing-kinetic experiments were started. Once the pH for the experiment was set, it was maintained by the automatic addition of acid (1 M HNO_3) or alkali (1 M NaOH) from the pH control unit. When the bacterial viable count became stable the oxygen saturation was approximately 5%. The purity of the chemostat culture during the experiments was monitored by daily Gram staining and culturing of samples at 37°C under aerobic, anaerobic, and microaerophilic conditions.

Establishment of batch cultures (flask system). Cultures of *H. pylori* were established with a set of 250-ml conical flasks each containing 98 ml of sterile brucella broth and 1 ml of fetal calf serum into which 1 ml of a standardized *H. pylori* (10^6 CFU/ml) culture was inoculated. The flasks were placed inside an orbital incubator at 37°C and were agitated at 150 rpm, and a filtered microaerophilic gas mixture was bubbled through the cultures at a rate of 150 ml/min. During the deceleration phase of growth (i.e., after about 16 h of incubation), when the cells were growing at a reduced rate compared to the rate at which they grew while they were in the midexponential phase, the test antibiotics were added at a concentration of 8× the MIC. Sixteen hours was chosen after calculation of the physiologic "growth cycle" showed deceleration of the growth rate at this time point under these experimental conditions.

Determination of bactericidal activity. Each antimicrobial agent was added in a pulse directly into the broth within the culture vessel to give a final concentration of 8× the MIC at the start of the experiments. After incubation of the organism-antibiotic mixture, a sample of 500 μl was removed from the chemostat vessel (or flasks) for determination of viable counts (numbers of CFU per milliliter) as described previously (31). The sample was serially diluted (1:10) in warm phosphate-buffered saline, and 20-μl volumes from each dilution were spread in triplicate onto Columbia blood agar plates. The inoculated plates were incubated at 37°C under microaerophilic conditions for 72 h before the colonies were counted. Only the organisms on plates yielding mean viable counts of at least 500 CFU/ml were counted, and the results were recorded. Samples were collected for viable count determinations immediately preceding the addition of

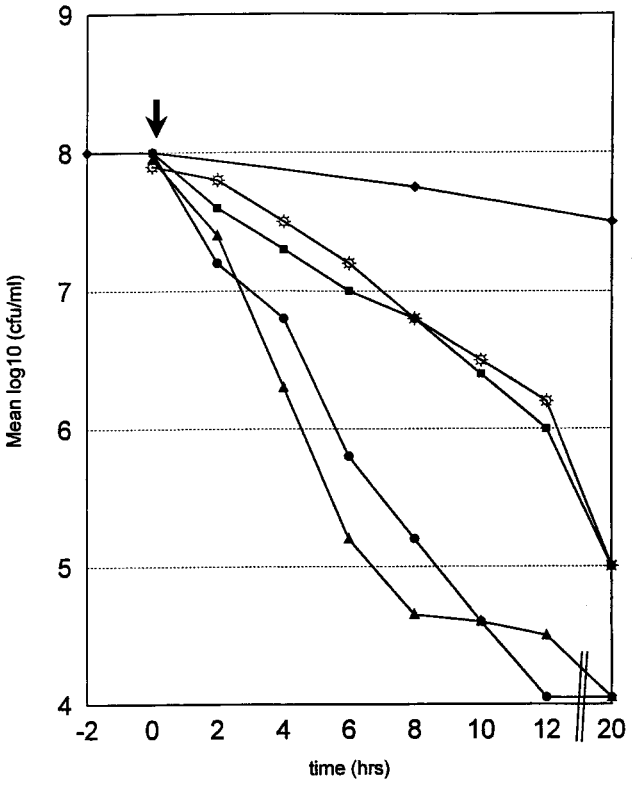

FIG. 2. Effect of pH on killing kinetics of amoxicillin (at 8× the MIC) in chemostat cultures for *H. pylori* NCTC 11637. ☼, pH 6.5; ■, pH 7; ●, pH 7.2; ▲, pH 7.4; ◆, washout; →, time of antibiotic addition.

antimicrobial agents to the chemostat vessel (or flasks) and at 2, 4, 6, 8, 10, 12, and 20 h. The presence of antimicrobial agents in the flasks (or chemostat) was monitored by a microbiological agar well diffusion assay as described previously (2). From the mean viable counts, killing and viability curves were determined, and from these curves the bactericidal activity of each agent was calculated. The bacterial inoculum at the start of the experiments was 8.0 ± 0.2 $\log_{10}$ CFU/ml. Each experiment was repeated three times.

RESULTS

MICs. The MICs of the antimicrobial agents for all the *H. pylori* isolates tested are presented in Table 1.

Chemostat. Figure 1 is a graphic representation of the chemostat killing curves for *H. pylori* NCTC 11637 and shows the activities of those agents that produce a fall in bacterial count of at least 3 $\log_{10}$ CFU/ml (≥99.9% killing) relative to that expected by washout of nondividing cells at 20 h. These bactericidal agents include amoxicillin, meropenem, imipenem, clarithromycin, and 14-OH-clarithromycin. Imipenem was the most rapidly bactericidal, with a decrease in the bacterial count of 4 $\log_{10}$ CFU/ml at 8 h. From 10 h onward after the addition of imipenem it was not possible to detect *H. pylori* by determination of viable counts from the chemostat broth, which became markedly less turbid. The bacteriostatic agents included ampicillin, cefixime, cefuroxime, cefotaxime, and ceftazidime, which gave decreases in bacterial count of <1.5 $\log_{10}$ CFU/ml, while azlocillin and piperacillin produced decreases of 2.5 $\log_{10}$ CFU/ml (data not shown).

Effect of pH on activities of antimicrobial agents. (i) Amoxicillin. Figure 2 shows the results of the effect of pH 6.5, 7.0, 7.2, and 7.4 on the activity of amoxicillin against *H. pylori* NCTC 11637. Amoxicillin was bactericidal at all pH values (≥99.9% killing). It was most rapidly cidal at pHs 7.4 and 7.2 with a decrease of >3 $\log_{10}$ CFU/ml at 10 h compared with a decrease of 3 $\log_{10}$ at pHs 7.0 and 6.5 at 20 h. The pH optima for killing by amoxicillin were 7.2 and 7.4.

(ii) Clarithromycin. Figure 3 shows the results of the effect of pHs 6.5 to 7.4 on the activity of clarithromycin against *H. pylori* NCTC 11637. Clarithromycin was bactericidal at pHs 7.0 to 7.4 and achieved killing of at least 3 $\log_{10}$ CFU/ml at 20 h (≥99.9% killing). It was, however, only bacteriostatic at pH 6.5 and caused a decrease in the bacterial count of 1 $\log_{10}$ CFU/ml at 20 h.

(iii) Combination of amoxicillin and clarithromycin. Figure 4 shows the killing curves for the combined activity of clarithromycin and amoxicillin against *H. pylori* NCTC 11637 at pHs 6.5 and 7.0. At both pHs the combination was bactericidal from 8 h onward. There was also greater cidal activity for the combination compared to the activities of the antibiotics individually at the same pHs. The cidal activity of amoxicillin at pH 6.5 was comparable to that at pH 7.0 (Fig. 4).

Batch culture (flask system). Figure 5 shows the killing curves for *H. pylori* NCTC 11637 in a batch-culture system. Amoxicillin, meropenem, imipenem, and clarithromycin were all bactericidal. Imipenem was still the most bactericidal agent, with a decrease of 3.5 $\log_{10}$ CFU/ml at 12 h, followed by meropenem, amoxicillin, and clarithromycin, with a decrease of 3 $\log_{10}$ CFU/ml at 20 h. 14-OH-clarithromycin was only bacteriostatic and gave a fall of 2.5 $\log_{10}$ CFU/ml. The other β-lactams were as bacteriostatic with the batch-culture system as with the chemostat (data not shown).

Other strains. For *H. pylori* NCTC 11916 and five clinical strains, imipenem was the most rapidly bactericidal agent, with a killing of at least 3 $\log_{10}$ CFU/ml at 20 h, and was the only

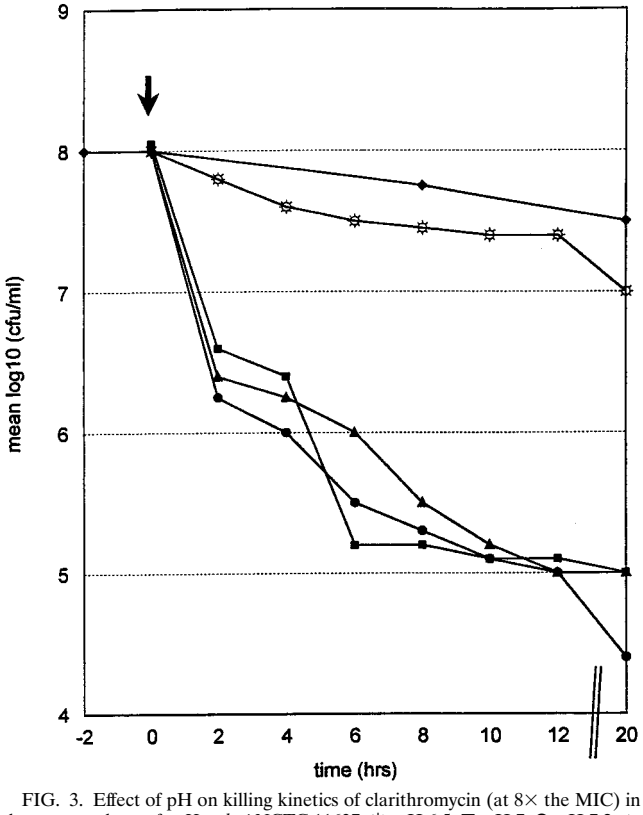

FIG. 3. Effect of pH on killing kinetics of clarithromycin (at 8× the MIC) in chemostat cultures for *H. pylori* NCTC 11637. ☼, pH 6.5; ■, pH 7; ●, pH 7.2; ▲, pH 7.4; ◆, washout; →, time of antibiotic addition.

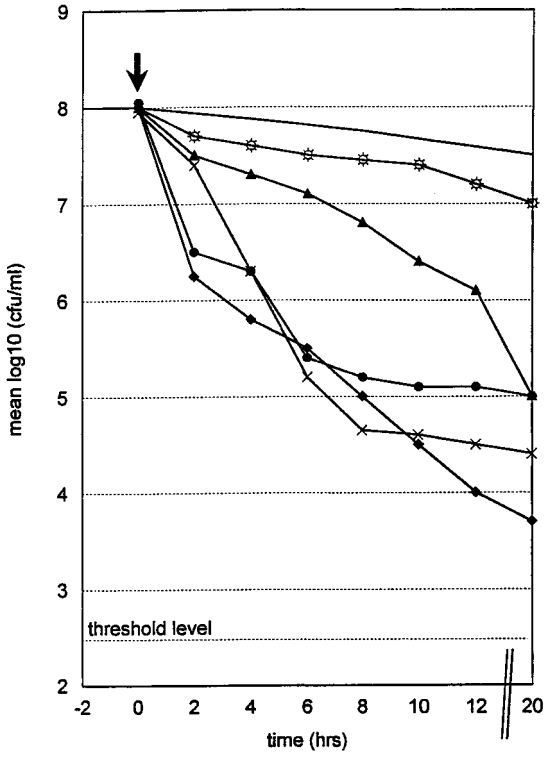

FIG. 4. Effect of pH on killing kinetics of clarithromycin, amoxicillin, and combinations (at 8× the MIC) in chemostat cultures for *H. pylori* NCTC 11637. ☼, clarithromycin at pH 6.5; ●, clarithromycin at pH 7.0; ▲, amoxicillin at pH 6.5; ×, clarithromycin and amoxicillin at pH 6.5; ◆, clarithromycin and amoxicillin at pH 7.0; —, washout; →, time of antibiotic addition.

agent that was bactericidal for two of the strains. For a further two strains (strains 3266 and AMMI) only imipenem and meropenem were bactericidal. For strain 18868 imipenem, amoxicillin, and clarithromycin were equally bactericidal at 20 h, while strain 2985 was equally killed at 20 h by imipenem, meropenem, and clarithromycin. For one strain (TIYO) clarithromycin was the only bactericidal agent, with the rest of the agents, including imipenem, remaining only bacteriostatic (Table 2).

DISCUSSION

We have compared the activities of 10 β-lactams, clarithromycin, and 14-OH-clarithromycin against slowly growing *H. pylori* NCTC 11637 in both the chemostat and batch cultures (flask system). The chemostat, which can grow bacteria at the rate set by the experimenter, is a good model for killing-kinetic studies. It may provide results which are more predictive of the in vivo situation in which bacteria have a more prolonged doubling time compared to that under in vitro conditions (28). The chemostat has been used previously for killing-kinetic studies (8, 32). However, we are unaware of any comparison of this system for killing-kinetic studies with cheaper traditional batch-culture methods. We have compared the two systems for killing-kinetic studies and have shown similar trends in the activities of β-lactam antibiotics and clarithromycin against slowly growing *H. pylori* NCTC 11637. In both systems, imipenem, meropenem, amoxicillin, and clarithromycin were bactericidal, while the remaining antimicrobial agents were bacteriostatic. Imipenem was the most rapidly bactericidal agent, although it was bactericidal to a lesser degree in the batch culture than in the chemostat. Also, 14-OH-clarithromycin, which was bactericidal in the chemostat, was bacteriostatic only in the batch culture (flask system).

The current search for agents with improved anti-*H. pylori* activities continues. No single agent give adequate eradication, and compliance is a problem with current multidrug regimens for the treatment of *H. pylori* infection (42). Clarithromycin has achieved the highest reported eradication rates of any monotherapy (34) and, like amoxicillin, has become an established part of combination therapy for *H. pylori* infection (33). The fact that imipenem and meropenem were bactericidal against most strains may be of interest in view of the current development of novel oral carbapenems (3, 40). Our results suggest that carbapenems may have a role in the therapy of *H. pylori*-associated disease. In vitro and gut pharmacokinetic studies of the newer oral carbapenems would need to be performed. Imipenem, for example, is widely distributed in body fluids and secretions including gastric juice, in which suprainhibitory concentrations have been found following intravenous administration (14, 39). A recent pilot study of imipenem monotherapy for 48 h has achieved good clearance but has failed to eradicate *H. pylori* from the gut (39). The short duration of therapy in this study makes comparison with longer-course regimens difficult. There is current interest in the use of intravenous antibiotics for the management of bleeding peptic ulcers in patients who are *H. pylori* positive. There is evidence to support the view that eradication of *H. pylori* from this group of patients prevents recurrence of the bleeding episode (1, 19, 26).

The study of the effect of pH changes on the activities of antimicrobial agents against microorganisms had been report-

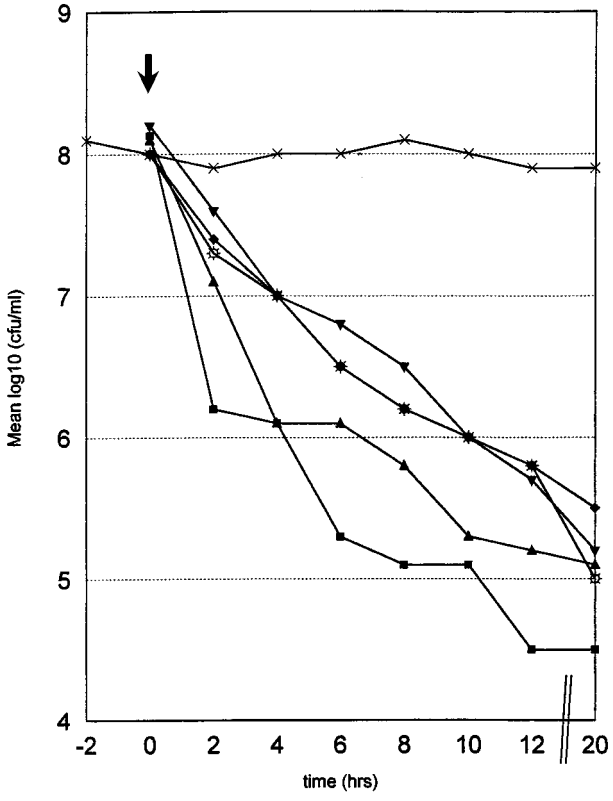

FIG. 5. Killing kinetics of antimicrobial agents (at 8× the MIC) at pH 7.0 in batch cultures for *H. pylori* NCTC 11637. ■, imipenem; ☼, meropenem; ▼, amoxicillin; ▲, clarithromycin; ◆, 14-OH-clarithromycin; ×, control; →, time of antibiotic addition.

ed previously for batch cultures (4, 17, 20). Changes in pH can affect the activities of antimicrobial agents as well as the expression of bacterial target sites. Our chemostat data showed that amoxicillin had pH optima for killing of *H. pylori* NCTC 11637 at 7.2 and 7.4. This is in agreement with previous reports (6, 37). This was not surprising since amoxicillin is an amphoteric agent which is less ionized at acidic pH. Clarithromycin was bactericidal at all pHs except pH 6.5. The activities of macrolides have been known to be decreased at acidic pH, but the degree to which they are affected varies between members of this group of agents (12). Clarithromycin has been reported to be bactericidal at acidic pH (13, 22), while others have reported a loss of activity of clarithromycin at acidic pH (6), a result similar to that of the present study.

The omeprazole-based combination therapy with clarithromycin and amoxicillin is now a regimen of choice for the treatment of *H. pylori* infection in Europe (27). The results of the present combination study show that at both pH 6.5 and pH 7.0, there was bactericidal activity which was greater than the activities of the individual agents at the same pH. Omeprazole facilitates secretion of antibiotics into the mucosa and increases tissue bioavailability (21). Previous studies have not demonstrated synergy between various combinations of omeprazole, amoxicillin, and clarithromycin in vitro (6, 30, 37).

In conclusion, the greater activity of imipenem compared with those of other β-lactams and clarithromycin should prompt further studies and the search for oral carbapenems with potential anti-*H. pylori* activity.

TABLE 2. Activities of antimicrobial agents against *H. pylori* strains

Strain	Activity[a]												
	Imipenem	Meropenem	Ampicillin	Amoxicillin	Clarithromycin	14-OH-clarithromycin	Cefixime	Cefuroxime	Cefotaxime	Piperacillin	Azlocillin	Ceftazidime
NCTC 11637	+	+	−	+	+	±	−	−	−	−	−	−
NCTC 11916	+	−	−	+	+	NT	−	−	−	−	−	−
18868	+	+	−	+	+	NT	−	−	−	−	−	−
2985	+	+	−	+	+	NT	−	−	−	−	−	−
3571	+	+	−	−	−	NT	−	−	−	−	−	−
3266	+	+	−	+	+	NT	−	−	−	−	−	−
2245	+	+	−	+	+	NT	−	−	−	−	−	−
ABBA	+	−	−	−	−	NT	−	−	−	−	−	−
AMMI	+	+	−	−	−	NT	−	−	−	−	−	−
HAWA	+	−	−	−	−	NT	−	−	−	−	−	−
TIYO	−	−	−	−	+	NT	−	−	−	−	−	−
KAL	+	+	−	+	+	NT	−	−	−	−	−	−

[a] +, cidal; −, static; ±, cidal in chemostat and static in batch cultures; NT, not tested.

REFERENCES

1. **Adamek, R. J., M. Freitag, W. Opferkuch, G. H. Ruhl, and M. Wegener.** 1994. I/V omeprazole/amoxycillin and omeprazole pre-treatment in *Helicobacter pylori* positive acute peptic ulcer bleeding. A pilot study. Scand. J. Gastroenterol. **29:**880–883.
2. **Anhalt, J. P.** 1985. Assays of antimicrobial agents in body fluid, p. 1009–1014. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
3. **Aoki, H., M. Sekiguchi, K. Akahane, Y. Tsutomi, H. Korenaga, T. Hayano, and I. Hayakawa.** 1995. A new oral carbapenem antibiotic. 3. Pharmacokinetics and safety in laboratory animals, abstr. F135, p. 136. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
4. **Barry, A. L., R. N. Jones, and C. Thornsberry.** 1988. In vitro activities of azithromycin (CP 62,993), clarithromycin (A-56268; TE-031), erythromycin, roxythromycin, and clindamycin. Antimicrob. Agents Chemother. **32:**752–754.
5. **Borsch, G., U. Mai, and K. M. Muller.** 1988. Monotherapy or polychemotherapy in the treatment of *Campylobacter pylori*-related gastroduodenal disease. Scand. J. Gastroenter. **23**(Suppl. 142)**:**101–106.
6. **Cederbrant, G., G. Kahlmeter, C. Schalen, and C. Kamme.** 1994. Additive effect of clarithromycin combined with 14-hydroxy-clarithromycin, erythromycin, amoxycillin, metronidazole, or omeprazole against *H. pylori*. J. Antimicrob. Chemother. **34:**1025–1029.
7. **Correa, P.** 1992. Human gastric carcinogenesis: a multistep and a multifactorial process. First American Cancer Society Award lecture on cancer epidemiology and prevention. Cancer Res. **52:**6735–6740.
8. **Cozens, R. M., E. Tuomanen, W. Tosch, O. Zak, J. Suter, and A. Tomasz.** 1986. Evaluation of the bactericidal activity of β-lactam antibiotics on slowly growing bacteria cultured in the chemostat. Antimicrob. Agents Chemother. **29:**797–802.
9. **DeCross, A. J., and B. J. Marshall.** 1993. The role of *Helicobacter pylori* in acid-peptic disease. Am. J. Med. Sci. **306:**381–392.
10. **Dooley, C. P.** 1993. Background and historical considerations of *Helicobacter pylori*. Gastroenterol. Clin. N. Am. **22:**1–4.
11. **Eurogast Study Group.** 1993. An international association between *Helicobacter pylori* infection & gastric cancer. Lancet **341:**1359–1362.
12. **Fiese, E. F., and S. H. Steffan.** 1990. Comparison of the acid stability of azithromycin and clarithromycin-A. J. Antimicrob. Chemother. **25**(Suppl. A)**:**39–47.
13. **Flamm, R. K., J. Beyer, S. K. Tanaka, and J. Clement.** 1996. Kill kinetics of antimicrobial agents against *H. pylori*. J. Antimicrob. Chemother. **38:**719–725.
14. **Geddes, A. M., and W. Stilles.** 1985. Imipenem: the first thienamycin antibiotic. Rev. Infect. Dis. **7**(Suppl. 3)**:**S353–S536.

15. **Gilbert, P.** 1985. The theory and relevance of continuous culture. J. Antimicrob. Chemother. **15**(Suppl. A)**:**1–6.
16. **Glupczynski, Y.** 1990. In vitro susceptibility of *Helicobacter pylori* to antibiotics & bismuth salts & the importance of acquired resistance to antibiotics in treatment failures of *Helicobacter pylori* infection, p. 49–58. *In* P. Malfertheiner and H. Ditschuneit (ed.), *Helicobacter pylori*, gastritis & peptic ulcer. Springer-Verlag, Berlin, Germany.
17. **Glupczynski, Y.** 1993. *In vitro* susceptibility testing of *H. pylori* to antimicrobial agents: basis for treatment of microbiologists' obsession? Zentbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **280:**227–238.
18. **Goodwin, C. S., P. Blake, and E. Blincow.** 1986. The MIC & MBC of antimicrobial agents and anti-ulcer agents against *Campylobacter pyloridis*. J. Antimicrob. Chemother. **17:**309–314.
19. **Graham, D. Y., K. S. Hepps, F. C. Ramirez, G. M. Lew, and Z. A. Saeed.** 1993. Treatment of *Helicobacter pylori* reduces the rate of rebleeding in peptic ulcer disease. Scand. J. Gastroenterol. **28:**939–942.
20. **Grayson, M. L., G. M. Eliopoulos, M. J. Ferraro, and R. C. Moellering.** 1989. Effect of varying pH on the susceptibility of *C. pylori* to antimicrobial agents. Eur. J. Clin. Microbiol. Infect. Dis. **8:**888–889.
21. **Gustavson, L. E., J. F. Kaiser, A. L. Edmonds, C. S. Locke, M. L. DeBartolo, and D. W. Schneck.** 1995. Effect of omeprazole on concentrations of clarithromycin in plasma and gastric tissue at steady state. Antimicrob. Agents Chemother. **39:**2078–2083.
22. **Hardy, D. J., C. W. Hanson, D. M. Hensey, J. M. Beyer, and P. B. Fernandes.** 1988. Susceptibility of *Campylobacter pylori* to macrolides and fluoroquinolones. J. Antimicrob. Chemother. **22:**631–636.
23. **Harris, A., and J. J. Misiewicz.** 1995. Hitting *Helicobacter pylori* for four. Lancet **345:**806–807.
24. **Holt, A., and D. Brown.** 1989. Antimicrobial susceptibility testing, p. 167–193. *In* P. M. Hawkey and D. A. Lewis (ed.), Medical bacteriology: a practical approach. IRL Press, Oxford, United Kingdom.
25. **International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans.** 1994. *Helicobacter pylori*, p. 177–240. *In* Schistosomes, liver flukes & *Helicobacter pylori*: views & expert opinions of an IARC working group on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer, Lyon, France.
26. **Labenz, J., and G. Borsch.** 1994. Role of *Helicobacter pylori* eradication in the prevention of peptic ulcer bleeding relapse. Digestion **55:**19–23.
27. **Maastricht Consensus Report.** 1997. Current European concepts in the management of *Helicobacter pylori*. Gut **41:**8–13.
28. **Maw, J., and G. G. Meynell.** 1968. The true division and death rate of *Salmonella typhimurium* in the mouse spleen determined with superinfecting phage P22. Br. J. Exp. Pathol. **49:**597–613.
29. **McNulty, C. A. M.** 1989. Bacteriological and pharmacological basis for the treatment of Campylobacter pylori infection. Gastroenterol. Clin. Biol. **13:** 96B–100B.
30. **Meyer, J. M., S. Ryu, S. L. Pendland, and L. H. Danziger.** 1997. In vitro synergy testing of clarithromycin and 14-hydroxyclarithromycin with amoxicillin or bismuth subsalicylate against *Helicobacter pylori*. Antimicrob. Agents Chemother. **41:**1607–1608.
31. **Miles, A. A., and S. S. Misra.** 1938. The estimation of the bactericidal power of the blood. J. Hyg. Camb. **38:**732–749.
32. **Millar, R. M., and J. Pike.** 1992. Bactericidal activity of antimicrobial agents against slowly growing *Helicobacter pylori*. Antimicrob. Agents Chemother. **36:**185–187.
33. **National Institutes of Health Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease.** 1994. *Helicobacter pylori* in peptic ulcer disease. JAMA **272:**65–69.
34. **Peterson, W. L., D. Y. Graham, B. Marshall, M. J. Blaser, R. M. Genta, P. D. Klein, C. W. Stratton, J. Drnec, P. Prokocimer, and N. Siepman.** 1993. Clarithromycin as monotherapy for eradication of *Helicobacter pylori*: a randomized double blind trial. Am. J. Gastroenterol. **88:**1860–1864.
35. **Rauws, E. A. J., and G. N. J. Tytgat.** 1990. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. Lancet **335:**1233–1235.
36. **Sharp, D.** 1995. Worms, spirals, flukes as carcinogens. Lancet **345:**403–404. (Commentary.)
37. **Sjostrom, J. E., and H. Larsson.** 1996. Factors affecting growth and antibiotic susceptibility of *Helicobacter pylori*: effect of pH and urea on the survival of a wild-type strain and a urease-deficient mutant. J. Med. Microbiol. **44:**425–433.
38. **Sung, J. J. Y., S. C. S. Chung, T. K. W. Ling, M. Y. Yung, V. K. S. Leung, K. W. Enders, K. K. Michael, A. F. B. Cheng, and K. C. Arthur.** 1995. Antibacterial treatment of gastric ulcers associated with *Helicobacter pylori*. N. Engl. J. Med. **332:**139–142.
39. **Sung, J. J. Y., S. C. S. Chung, S. W. Hosking, R. C. Y. Chan, T. K. W. Ling, M. Y. Yung, A. F. B. Cheng, and A. K. C. Li.** 1995. Mucosal pharmacokinetics and pilot study of short course of parenteral imipenem in the eradication of *Helicobacter pylori*. J. Gastroenterol. Hepatol. **10:**66–69.
40. **Tanaka, M., M. Hohmura, T. Nishi, K. Sato, and I. Hayakawa.** 1997. Antimicrobial activity of DU-6681a, a parent compound of novel oral carbapenem DZ-2640. Antimicrob. Agents Chemother. **41:**1260–1268.
41. **Tempest, D. W.** 1970. The continuous cultivation of microorganisms. Theory of the chemostat. Methods Microbiol. **2:**259–276.
42. **Walsh, J. H., and W. L. Peterson.** 1995. The treatment of *H. pylori* infection in the management of peptic ulcer disease. N. Engl. J. Med. **333:**984–991.
43. **Wotherspoon, A. C., C. Doglioni, T. C. Diss, L. Pan, A. Moschini, M. Boni, and P. G. Isaacson.** 1993. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet **342:**575–577.