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Basis for the Selective Antibacterial Activity In Vitro of Proton Pump Inhibitors against *Helicobacter* spp.

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Proton pump inhibitors of the benzimidazole type exert a specific antibacterial activity against *Helicobacter pylori* in vitro. In the present study, the basis for this selectivity was investigated, and in particular, various factors affecting the in vitro antibacterial activity of sulfide analogs of benzimidazoles were studied. Upon preincubation of omeprazole for a period of up to 72 h in a buffer at pH 7, a product was formed that was bactericidal for *H. pylori* but had no effect on urease activity. Sulfide constitutes the main end product of degradation. The sulfide analog of omeprazole (H 168/22) exerted a bactericidal activity specifically against both resting (in buffer) and growing (in broth) *Helicobacter* spp., and time-kill in buffer at pH 5 was enhanced compared to that at pH 7. There was no or very low covalent binding of ^{3}H-labeled H 168/22 to *Helicobacter* spp. or to other gram-negative and gram-positive bacteria. In the presence of fetal calf serum (FCS) under the same conditions, binding was only slightly lowered while the killing activity was markedly reduced, indicating a probably nonspecific interaction with proteins and/or protection of bacterial target(s) by FCS. Addition of H 168/22 (four times the minimum bactericidal concentration [MBC]) to exponentially growing *H. pylori* immediately stopped growth, and after an incubation period of 20 h viable counts were reduced by >7 $\log_{10}$. One-hour exposure of *H. pylori* to the drug followed by repeated washing retarded growth by about 2 h, indicating that the effect is reversible after short-term exposure. MICs and MBCs of various sulfide structures were lower than those obtained in broth after the addition of the corresponding sulfoxide. Thus, the MBC of the sulfide structure of omeprazole against 140 clinical isolates of *H. pylori* ranged from 8 to 32 μg/ml, compared to an MBC of omeprazole of 32 to 128 μg/ml. A similar potency was also recorded against other helicobacters. In conclusion, formation of sulfides of benzimidazoles in culture media is the reason for the selective antibacterial effect against *H. pylori*. The sulfides rapidly exerted a reversible antibacterial activity, which was specific against both resting and growing *Helicobacter* spp. without any covalent protein binding.

Omeprazole and structural benzimidazole analogs are potent inhibitors of gastric acid secretion. After proton-dependent activation (3, 4), they are transformed into the sulfenamide form, which inhibits the acid pump, H^+, K^+-ATPase, in the parietal cell. Furthermore, benzimidazole proton pump inhibitors (PPIs) have been shown to exert a specific antibacterial activity against *Helicobacter pylori* in vitro (9, 15, 21, 23). However, when the compound was given at more than 100 times the therapeutic dose, it failed to eradicate *Helicobacter felis* in rats (12). At therapeutic doses the plasma concentrations of omeprazole are well below those needed for antibacterial action in vitro, i.e., around 100 μM (36 μg/ml). Although it is difficult to completely rule out the possibility that omeprazole exerts a direct antibacterial effect in vivo, this seems highly unlikely. Amoxicillin, at sufficiently high doses given for 2 to 4 weeks, eradicates *H. felis* infection in rats to a significant degree, and simultaneous omeprazole treatment potentiates this effect. The most plausible interpretation is that the synergistic effect with omeprazole and antibiotics in vivo, which is the standard therapy for eradication of *H. pylori*, is due to the fact that the increase in gastric pH caused by omeprazole provides a more favorable environment for growth of *H. pylori* and/or for antibiotic action (22). Neither the mechanism underlying the antibacterial effect seen in vitro nor the structural prerequisite for the selectivity has been elucidated in detail. We have previously reported that this bactericidal activity is dependent on the experimental conditions used (21) and that the activity exerted in buffer is due to the formation of the sulfenamide structure, which is not specific to *Helicobacter* spp. The sulfenamide interacts with SH groups accessible in bacteria, and in vitro it affects both the urease (11, 17) and alcohol dehydrogenase (20) activities associated with *H. pylori*. However, a derivative of lansoprazole, AG-2000, has been shown to inhibit the growth of *H. pylori* without affecting urease activity (18). It has also been reported that omeprazole interacts with a P-type ATPase in the bacterium, an enzyme suggested to be essential for growth and survival (14). Ge et al. (5) have shown that another P-type ATPase involved in copper export is not essential for *H. pylori*. However, PPIs have no effect on the F ATPase activity of *H. pylori* (1). In this study we demonstrate that in broth culture, structures other than sulfenamide are important for bactericidal activity against *Helicobacter* spp. In the presence of β-mercaptoethanol or fetal calf serum (FCS) in buffer or in broth at neutral pH, omeprazole as well as other sulfoxide analogs will predominantly form the sulfide analog. Therefore, it was hypothesized that it is this analog that exerts the specific antibacterial activity against *Helicobacter* spp., and it is also the minimum bactericidal concentration (MBC) of this analog that has been determined.

The aim of the work reported here was to show that conversion products of omeprazole other than the sulfenamide are involved in the selective bactericidal activity against *H. pylori* and to characterize the antibacterial activity exerted by the sulfide analog of PPIs in vitro.

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TABLE 1. MBCs of the sulfide structure of omeprazole (H 168/22) and bactericidal activity against different bacterial strains[a]

Microorganism	Strain	MBC (μg/ml)	Reduction in viable count (log_{10} CFU/ml)
H. pylori	ATCC 43504	32	>7
H. felis	CS1	16	>5
H. mustelae	ATCC 43772	32	ND[c]
Helicobacter muridarum	ATCC 29262	32	>6
Helicobacter cinaedi	CCUG 18818T	16	ND
C. hyointestinalis	CCUG 27631	>256	<1
Campylobacter jejuni	CCUG 11284	>256	<1
E. coli	K-12	>256	<1
B. subtilis	CCUG 163	>256	<1
S. aureus	CCUG 1800T	>256	<1
P. aeruginosa	CCUG 551T	>256	1
P. vulgaris	CCUG 6327	>256	1
Klebsiella pneumoniae	CCUG 225	>256	<1
H. pylori[b]	Clinical isolates	8–32	ND

[a] MBCs were determined in brucella broth with 10% FCS (pH 7.0). Reduction in viable counts was determined during 5 h of incubation in 100 mM phosphate-citrate buffer, pH 5, with 128 μg of H 168/22 per ml.
[b] MBCs for 140 clinical isolates from duodenal ulcer patients.
[c] ND, not determined.

MATERIALS AND METHODS

Microorganisms. The bacterial genera, species, and strains used in this study are listed in Table 1. Stock cultures were stored at −70°C in brucella broth (Difco) with 10% FCS, pH 7.0, supplemented with 20% glycerol. The type strain of *H. pylori* selected for this study was ATCC 43504 from the American Type Culture Collection (Rockville, MD), and 140 isolates of *H. pylori* from duodenal ulcer patients were also studied. *H. felis* CS1 was a gift from A. Lee, Sydney, Australia (13, 19). Strains with the prefix CCUG are from the Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Sweden.

Media and growth conditions. The solid medium for *Helicobacter* spp. and *Campylobacter* spp. was Columbia blood agar, which consisted of 42.5 g of Columbia Agar Base II (Oxoid) per liter, 15 g of Bacto Agar (Oxoid) per liter, 7% horse blood, and 1.0% IsoVitaleX (BBL Microbiology System) (pH 7.3 ± 0.2). All other species were grown on Luria agar (LA) (10 g of Bacto Peptone [Difco], 5 g of yeast extract [Difco], 10 g of NaCl, and 15 g of Bacto Agar [Difco] in 1,000 ml of distilled H_2O). The growth medium for determination of MICs and MBCs was brucella broth (pH 7.0; Difco) supplemented with 10% FCS. *Helicobacter* spp. and *Campylobacter* spp. were grown at 37°C in an automatic CO_2-O_2 incubator (Forma Scientific) under microaerobic conditions (85% N_2, 10% CO_2, and 5% O_2). All other organisms were grown at 37°C under aerobic conditions.

Chemicals. Dimethylsulfoxide (DMSO) from Merck (Darmstadt, Germany) was used to dissolve analogs of benzimidazoles, which were synthesized by Astra Hässle AB. Structures of the sulfide analogs of the PPIs lansoprazole, omeprazole, pantoprazole, and pariprazole are shown in Table 2.

Determination of urease activity. Urease activity was determined by the application of 20 μl of the bacterial suspension onto urea-agar plates, which consisted of 25 g of urea agar base (Diagnostica, Merck) in 1,000 ml of H_2O supplemented with 5 ml of a filter-sterilized 40% urea solution. The plates were read after 1 h of incubation at room temperature, and the zones where the color of the pH indicator (phenol red) had changed were measured.

Determination of bactericidal activity and time-kill studies in buffer. Bacteria grown on Columbia blood agar or LA plates were collected and washed in phosphate-buffered saline (PBS) by centrifugation at 1,700 × *g* for 10 min at room temperature. The bacteria were then resuspended in 1.0 ml of sterile 100 mM citrate-phosphate buffer (pH 5 or 7) to a cell density of 10^7 to 10^8 CFU/ml. Compounds dissolved and diluted in DMSO or vehicle were then added. Viable counts (CFU per milliliter) for *Helicobacter* spp. and *Campylobacter* spp. were determined on blood agar plates after washing and serial dilutions in PBS (pH 7.2) after 3 days of incubation at 37°C. Viable counts for the other bacteria studied were determined on LA plates after 24 h of incubation at 37°C under aerobic conditions.

Preincubation of the sulfoxide structure of omeprazole under different conditions. The antibacterial activity exerted by preincubated omeprazole was determined after incubation of the drug at 128 μg/ml (two times the MBC) in 100 mM phosphate-citrate buffer at pH 7, with and without 1.43 mM β-mercaptoethanol, and in brucella broth plus 10% FCS at pH 7. After different preincubation periods at room temperature, 1-ml samples were withdrawn, and 10 μl of a freshly prepared bacterial suspension of *H. pylori* (10^7 CFU/ml) in 100 mM phosphate-citrate buffer at pH 7 was added. The antibacterial activity in the samples was estimated by determination of numbers of surviving bacteria after 2 and 5 h of exposure.

Determination of MICs and MBCs. MICs and MBCs of structural analogs of benzimidazoles for *Helicobacter* spp., *Campylobacter* spp., and other species studied were determined in microtiter plates as described previously (20). The compounds were tested in the range of 0.5 to 256 μg/ml. The MIC and the MBC were defined as the lowest concentration of the compound giving complete inhibition of growth, as indicated by optical density at 560 nm and by the presence of <10 colonies per spot of replicated samples, respectively.

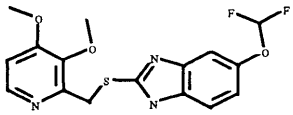
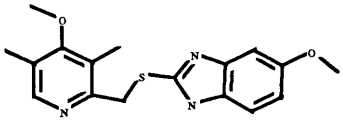
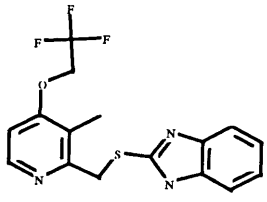
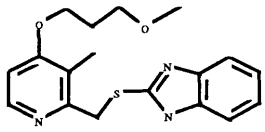
Binding of ^{3}H-labeled H 168/22 to *H. pylori* and other bacteria. The bacteria, grown as described above, were harvested and washed once in PBS and resuspended in 100 mM citrate-phosphate buffer at pH 5 or 7. The A_{560} of the bacterial suspension was adjusted to 2.5 in a spectrophotometer (Shimadzu UV-120-01). ^{3}H-labeled H 168/22 (20 μCi in 25 μl) was added to 1 ml of bacterial suspension kept in a shaking water bath at 37°C to a final concentration of 370 μM (four times the MBC). Binding was stopped after different times by centrifugation at 1,400 × *g* for 10 min, after which the bacteria were washed five times in 100 mM citrate-phosphate buffer. The remaining bacterial pellet was dissolved in 300 μl of 0.5 mM NaOH, and the amount of protein was determined by using a DC protein assay kit (Bio-Rad, Richmond, Calif.) with bovine serum albumin as the standard. The radioactivity in 50 μl was measured in a scintillation counter (Rackbeta 1219; LKB Wallac OY, Turku, Finland) after the addition of 3 ml of acidified scintillation cocktail (Ready Safe; Beckman). Binding levels were expressed as disintegrations per minute per microgram of protein.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Labeled material was treated and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (20).

RESULTS

Antibacterial and antiurease activity after preincubation of omeprazole under various conditions. The results in Fig. 1 show that, after preincubation of omeprazole under different conditions, bactericidal activity against *H. pylori* gradually developed. In buffer at pH 7.0 (Fig. 1A) the antibacterial activity increased with the preincubation time, and after 72 h of preincubation a 2-h exposure of the bacteria to the pretreated

TABLE 2. Sulfide structural analogs of PPIs and their MBCs against *H. pylori* ATCC 43504

Compound	Structure	MBC (μg/ml)
H 258/28[a]		64
H 168/22[b]		32
H 225/18[c]		8
H 295/43[d]		1

[a] Analog of pantoprazole (MBC, >128 μg/ml).
[b] Analog of omeprazole (MBC, 64 μg/ml).
[c] Analog of lansoprazole (MBC, 16 μg/ml).
[d] Analog of pariprazole (MBC, 4 μg/ml).

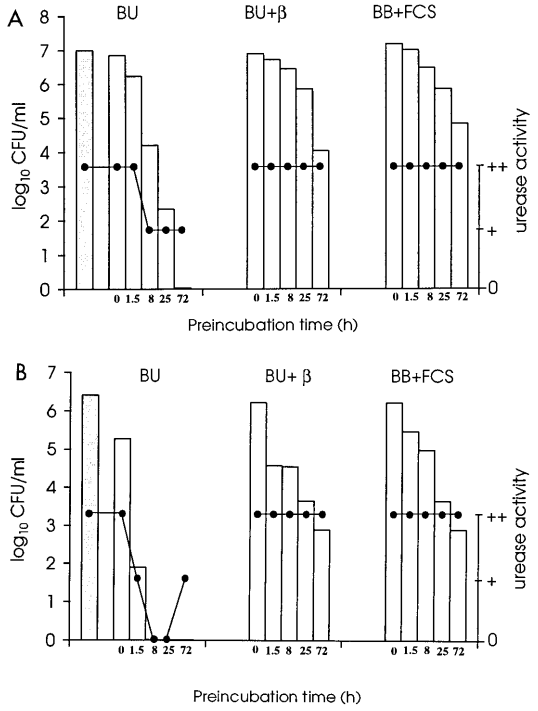


FIG. 1. Bactericidal activity of omeprazole (H 168/68), 128 μg/ml, preincubated in 100 mM citrate-phosphate buffer, pH 7.0, (BU) in the presence of 1.43 mM β-mercaptoethanol (BU+β) and in brucella broth plus 10% FCS, pH 7, (BB+FCS) for different periods at room temperature. The number of surviving *H. pylori* ATCC 43504, expressed as the number of CFU/ml, was determined after 2 h (A) and 5 h (B) of exposure to the preincubated samples at 37°C in buffer at pH 7.0. At the start there were 10^7 CFU/ml, and the survival in 1.25% DMSO, the vehicle, was measured (□). Urease activity (●) in 20-μl samples was determined on urea-agar plates by measuring the sizes of zones showing a color change, with phenol red as a pH indicator. ++, high activity; +, low activity; 0, no activity.

sample of omeprazole reduced the number of surviving bacteria by 6 $\log_{10}$. Furthermore, the urease activity was also reduced. In the presence of a high concentration of β-mercaptoethanol, which acts as a scavenger of the sulfenamide structure and directs the reaction towards the formation of the sulfide, bactericidal activity again developed, but the urease activity was not affected. When the bacteria were exposed for 5 h to omeprazole preincubated in buffer (Fig. 1B), viability was further reduced compared to that after 2 h of exposure and a complete inhibition of the urease activity was observed. After 72 h of preincubation, the urease activity appeared again, but there was still potent antibacterial activity present (Fig. 1B), indicating the formation of an antibacterial product, most probably the sulfide, which is the end product after the complete conversion of the sulfenamide (2). An identical experiment with pariprazole (a sulfoxide with a much shorter half-life than omeprazole, thus generating its sulfide faster) showed that the half-life of the compound determines the velocity with which the bactericidal product appears (data not shown). The time-kill in the presence of β-mercaptoethanol or FCS was slower than with the sulfenamide structure in buffer alone and was more pronounced after a longer exposure time (Fig. 1B).

MICs and MBCs of sulfides of PPIs. MICs and MBCs previously reported for PPIs (21) were determined by addition of the sulfoxide structure to broth medium. As discussed above, this results in the generation of a number of different intermediates, the concentrations of which will vary with pH and time, depending on the half-life of the sulfoxide added (21). In order to discover whether there is a structure-activity relationship between sulfide structures of PPIs in vitro against *H. pylori*, we determined the MICs and MBCs for these compounds. The results in Table 2 show that the sulfide structures given alone were more potent than the mixture of intermediates obtained after the addition of the corresponding sulfoxide (21). This was valid for all PPIs, and the potencies for the sulfide structures were arranged in the same order as that for the corresponding sulfoxides (21). The difference in potency indicates that it should be possible to construct a structure-activity relationship between sulfides of benzimidazoles.

Factors affecting the binding and antibacterial activity of H 168/22. In order to study whether the antibacterial activity of H 168/22 was accompanied by binding to the bacteria, studies were performed with ^{3}H-labeled H 168/22 in buffer at pH 5 and 7. The results in Fig. 2A show that binding at both pH 5 and 7 was poor (0.2% to 0.5% of total radioactivity), and only slightly higher binding was seen at the lower pH. The antibacterial activity at pH 7 was lower than that at pH 5 (Fig. 2B). At pH 5 the reduction in the number of surviving bacteria was 8 $\log_{10}$ after an incubation period of 8 h. In buffer without the drug at pH 5, a reduction of 1 $\log_{10}$ was observed. The binding and bactericidal activity of H 168/22 were affected very little by β-mercaptoethanol, a known scavenger of sulfenamide. However, FCS almost entirely abolished the bactericidal activity during 4 h of incubation, but binding was only reduced by approximately 50% (Fig. 3). The binding of H 168/22 was even lower in brucella broth plus 10% FCS at pH 7.0 (data not shown), probably due to nonspecific binding to proteins, just as in buffer supplemented with serum (Fig. 3).

Electrophoretic pattern of *H. pylori* proteins after incubation with H 168/22. After incubation with ^{3}H-labeled H 168/22 in 100 mM citrate-phosphate buffer (pH 6.0) for 1 h, 10% to 17% of loaded radioactivity was recovered in the gel. There was no binding of H 168/22 to any particular protein in *H. pylori* (data not shown).

Binding and antibacterial activity of H 168/22 against other bacteria. In order to study the selectivity of binding and of the bactericidal activity of H 168/22 for *H. pylori*, the above experiments were repeated with *Campylobacter hyointestinalis*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. As with *H. pylori*, the level of binding was low and no differences in binding were observed between gram-negative and gram-positive bacteria (data not shown). The antibacterial activity at pH 5 was determined after 5 h of incubation. This incubation with

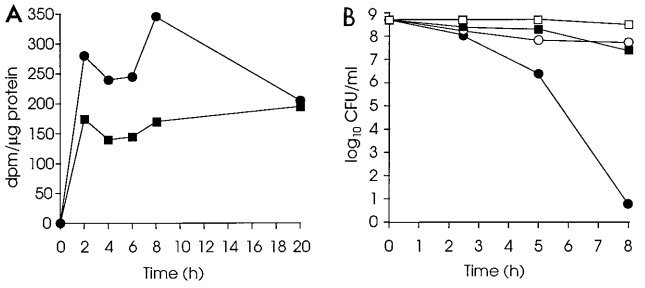


FIG. 2. Binding of ^{3}H-labeled sulfide of omeprazole, H 168/22 (128 μg/ml [four times the MBC]), to *H. pylori* ATCC 43504 in 100 mM citrate-phosphate buffer (A) and bactericidal activity of H 168/22 (B) at pH 5 (●) and pH 7 (■). The number of surviving bacteria in the presence of 1.25% DMSO without the drug is shown at pH 5 (○) and at pH 7 (□). Samples were withdrawn after different periods of incubation at 37°C. Binding was expressed as disintegrations per minute per μg of protein, and the number of surviving bacteria was expressed as the number of CFU per milliliter.

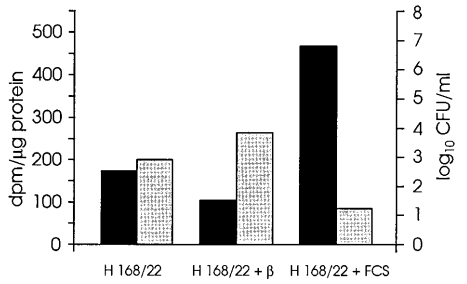


FIG. 3. Effect of β-mercaptoethanol (β) (1.43 mM) and FCS (10%) on binding of ^{3}H-labeled sulfide of omeprazole (H 168/22) at 128 μg/ml (four times the MBC) to *H. pylori* ATCC 43504 and bactericidal activity in 100 mM citrate-phosphate buffer (pH 5). Bound H 168/22 (given as disintegrations per minute per microgram of protein [□]) and viable counts (given as the number of CFU per milliliter [■]) were determined after 4 h of incubation at 37°C. At the start there were 4×10^7 CFU/ml.

the vehicle alone at pH 5 affected the viability of some of the bacteria studied, and therefore figures given in Table 1 show the additional compound-mediated reduction in viability. The results show reductions in viable counts for *Helicobacter* spp. in the range of 5 $\log_{10}$ to 6 $\log_{10}$. Among the other bacteria studied, a reduction in viability of only 1 $\log_{10}$ was obtained for *Proteus vulgaris* and *Pseudomonas aeruginosa*. However, other species were not at all affected by the drug, indicating that the bactericidal activity exerted by H 168/22 was selective for *Helicobacter* spp.

MICs and MBCs of H 168/22 for different bacteria. Table 1 shows that only *Helicobacter* spp. were susceptible to H 168/22 in brucella broth with 10% FCS (pH 7.0) and that members of other genera were not affected by the drug at 256 μg/ml under the conditions studied. MICs and MBCs of the sulfide structure of omeprazole against 140 clinical isolates of *H. pylori* ranged from 8 to 32 μg/ml, the MIC often being half the recorded MBC. Corresponding figures for other *Helicobacter* spp. ranged in the same interval, and both gastric and nongastric helicobacters were susceptible.

Effect of H 168/22 on exponentially growing *H. pylori* in brucella broth plus 10% FCS at pH 7.0. Addition of H 168/22 (four times the MBC) to an exponentially growing culture of *H. pylori* (4×10^7 CFU/ml) led rapidly to a complete stop in growth (Fig. 4), and after 20 h of incubation there were no viable bacteria. After 1 h of exposure and rapid drug removal by repeated washing there was an inhibition of growth that persisted for about 2 h before normal growth was again resumed.

DISCUSSION

In this report we show that sulfides of benzimidazole PPIs exert a selective antibacterial activity in vitro against *Helicobacter* spp. We have further shown that upon preincubation of omeprazole or pariprazole for a period of up to 72 h in a buffer at pH 7, a product was formed that was bactericidal for *H. pylori* but had no effect on urease activity. After addition of a PPI (sulfoxide) to a broth medium the sulfide will be generated, and the amount formed and the rate at which this occurs depend on the half-life of the sulfoxide compound, the pH, and the presence of SH groups. The sulfide is not subject to the same inherent reactivity as other intermediates of benzimidazoles are (2) and will therefore be the main end product obtained in broth and the dominating molecular species determining the MICs and MBCs. We therefore characterized the antibacterial activity exerted by the sulfide structure of omeprazole in vitro. Binding of radiolabeled sulfide of omeprazole, H 168/22, to all bacteria studied was low (0.2% to 0.5% of total radioactivity) but a little higher in buffer at pH 5 compared to that at pH 7. The corresponding binding of radiolabeled omeprazole at pH 5 was 40% to 50% of total radioactivity (21). No covalent binding to any particular protein of *H. pylori* was detected, and addition of β-mercaptoethanol, a known scavenger of sulfenamide, did not affect binding and bactericidal activity, showing that the sulfenamide intermediate was not generated or involved in binding and in bacterial killing. However, in the presence of FCS, the bactericidal activity was markedly reduced, indicating, together with the small reduction in binding, a probably nonspecific interaction with proteins and/or protection of bacterial target(s) by FCS. The viabilities of many bacteria were reduced during incubation for 5 h without exposure to the drug. However, in the presence of 128 μg of H 168/22 per ml, the viabilities of *H. pylori*, *H. felis*, and *Helicobacter mustelae* were further reduced by 5 $\log_{10}$ to 6 $\log_{10}$ compared to a reduction of only 1 $\log_{10}$ for *P. vulgaris* and *P. aeruginosa*, the most sensitive of all other bacteria tested. These results indicate that the bactericidal activity exerted in buffer and broth by the sulfide is specific and restricted to *Helicobacter* spp. MBCs for 140 clinical isolates of *H. pylori* from duodenal ulcer patients ranged in the interval of 8 to 32 μg/ml, and this was similar to what was found for other helicobacters studied. The MBCs for sulfides of benzimidazoles were lower than the MBCs obtained for their corresponding sulfoxides. Furthermore, sulfides of PPIs show different MBCs, and we have indications that a structure-activity relationship in vitro may be constructed (unpublished data).

In comparison with the potency of antibiotics used in successful therapies against *H. pylori* (6), e.g., amoxicillin, metronidazole, and clarithromycin, the potency of nonoptimized structures of sulfides of benzimidazoles is much lower, but sulfides of benzimidazoles are not affected by pH and bacterial growth as are some of these antibiotics (22). Furthermore, the time-kill for H 168/22 was relatively short compared to the antibiotics and no spontaneous mutants resistant to sulfides of benzimidazoles have been isolated (data not shown).

The mechanism of action of the sulfide compounds is not known. They were bactericidal on both resting and growing bacteria and were active at both pH 5 and 7, indicating that the target is a bacterial structural component rather than a protein dependent on bacterial growth for its expression. Addition of

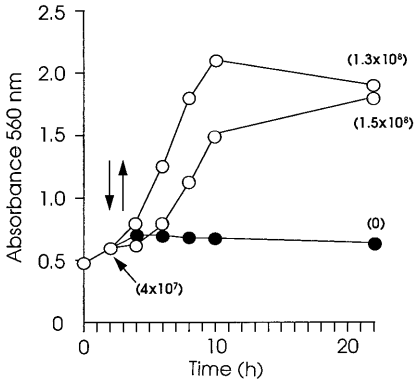


FIG. 4. Effect of sulfide of omeprazole (H 168/22) (128 μg/ml [four times the MBC]) on exponentially growing *H. pylori* ATCC 43504 in brucella broth plus 10% FCS, pH 7.0 (●) and retardation of growth after 1 h of exposure to the drug (between arrows) and repeated washing (◎). Growth without exposure to H 168/22 is also shown (○). Growth was monitored at A_{560}, and viable counts (CFU/ml) were determined at the start and after 20 h of incubation.

H 168/22 to growing *H. pylori* resulted in an immediate stop in growth and a complete killing of the bacteria in the 20-h incubation period. However, our results also suggest that the effect is reversible, at least after short-term exposure to the compound. This was further corroborated by the lack of covalent binding to proteins in *H. pylori*. The lipophilic nature of this group of compounds would also suggest an interaction with the cell membrane. Thus, the membrane composition in *H. pylori* may be specifically susceptible to agents of this character (unpublished data). Furthermore, emulsifying agents, which interact with membranes, have been shown to be much more potent against *Helicobacter* spp. in vitro than against other bacteria (10), a fact that may be due to the unique lipopolysaccharide and membrane phospholipid compositions of *H. pylori* (8, 16). The membrane composition of *H. pylori* has indeed also been shown to be an important chemotaxonomic marker for this species (7, 10).

In conclusion, our data show that sulfides of benzimidazoles exert a bactericidal activity that is specific for *Helicobacter* spp. The sulfide effect is the most likely basis for the previously observed selectivity of omeprazole for *Helicobacter* spp. Apart from the selectivity, the pharmacological profile in vitro for this group of compounds also has other interesting features, e.g., activity at pH 5 to pH 7 on resting and growing bacteria without any spontaneous development of resistance.

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