ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1996, p. 302–306 Vol. 40, No. 2
0066-4804/96/$04.00+0

Susceptibility of *Helicobacter pylori* to Bactericidal Properties of Medium-Chain Monoglycerides and Free Fatty Acids

BRYON W. PETSCHOW,* ROSANNE P. BATEMA, AND LORNA L. FORD

Bristol-Myers Squibb Co., Mead Johnson Research Center, Evansville, Indiana 47721

Received 17 July 1995/Returned for modification 29 September 1995/Accepted 15 November 1995

Previous studies have shown that various short- and medium-chain free fatty acids (FFAs) and their corresponding monoacylglycerol esters (MGs) have antibacterial activity in vitro against primarily gram-positive bacteria. More recent studies have also shown that the growth of *Helicobacter* spp. is inhibited by linoleic acid and arachidonic acid. The purpose of the present study was to evaluate the susceptibility of *Helicobacter pylori* to the in vitro bactericidal properties of medium-chain MGs and FFAs. Incubation of *H. pylori* with saturated MGs, ranging in carbon chain length from $C_{10:0}$ to $C_{14:0}$, at 1 mM caused a 4-log-unit or greater reduction in the number of viable bacteria after exposure for 1 h. Lower levels of bactericidal activity were observed with $C_{9:0}$, $C_{15:0}$, and $C_{16:0}$ MGs. In contrast, lauric acid ($C_{12:0}$) was the only medium-chain saturated FFA with bactericidal activity against *H. pylori*. The MGs and FFAs were bactericidal after incubation for as little as 15 min at neutral or acidic pHs. Higher levels of MGs and FFAs were required for bactericidal activity in the presence of higher amounts of protein in liquid diets. We also found that the frequency of spontaneous development of resistance by *H. pylori* was higher for metronidazole and tetracycline (10^{-5} to 10^{-6}) than for $C_{10:0}$ MG, $C_{12:0}$ MG, and $C_{12:0}$ FFA ($<10^{-8}$). Collectively, our data demonstrate that *H. pylori* is rapidly inactivated by medium-chain MGs and lauric acid and exhibits a relatively low frequency of spontaneous development of resistance to the bactericidal activity of MGs. Further studies are needed to establish whether MGs may be useful either alone or with other known therapeutic agents in the management of *H. pylori* infections in humans.

A large body of evidence now exists to support the role of *Helicobacter pylori* as the etiologic agent of active chronic gastritis and peptic ulcer disease and as a risk factor for development of gastric carcinoma. Treatment with single agents that have in vitro activity against *H. pylori* appears to clear infection in a large percentage of patients, but the rate of recurrent infection is high (21, 25). Greater success has been achieved with combination therapy, but recurrence of infection remains a common problem. Other problems reported for current eradication therapies include treatment side effects and noncompliance (12, 24), uncertainty concerning eradication rates (24), and development of resistance to antimicrobial agents by *H. pylori* (11, 21). Such problems might explain why the use of antimicrobial agents to eradicate *H. pylori* has not become a more accepted practice in the routine management of ulcer patients despite the strong evidence for the pathogenic role of *H. pylori* in gastritis and peptic ulcer disease (1, 4, 22, 24).

A number of free fatty acids (FFAs) and their corresponding esters are known to have potent antibacterial and antiviral activities (2, 5, 10, 14–17, 19, 20, 23). Previous reports indicate that such bactericidal activity is associated with FFAs and monoacylglycerol esters (MGs) but not di- or triglycerides, is greatest for compounds having 12 carbon atoms, and is typically greater for gram-positive bacteria than for gram-negative bacteria (5, 15–17, 23). Although the mechanism by which MGs and FFAs exert their antibacterial activity has not been defined, disruption of the cell membrane permeability barrier and inhibition of amino acid uptake have been suggested (18, 28). Preliminary observations in our laboratory showed that certain medium-chain MGs and FFAs were bactericidal for *H. pylori* and suggested that such compounds may be useful agents for the treatment of *H. pylori* infections in patients with gastritis and ulcer disease.

In the study presented in this report we evaluated the in vitro bactericidal activity of a number of FFAs and MGs against several strains of *H. pylori* under various experimental conditions. We also compared the frequencies of spontaneous development of resistance to several MGs and antibiotics among different laboratory strains of *H. pylori*, since resistance to antimicrobial agents remains an important problem for the available *H. pylori* treatment strategies.

MATERIALS AND METHODS

Bacterial strains. Human isolates of *H. pylori* (ATCC 43629, 43579, 49503, and 43526) were obtained from the American Type Culture Collection (Rockville, Md.). Stock cultures of *H. pylori* were grown for 3 to 5 days on horse blood agar plates with gonococcus heart infusion enrichment (Remel, Lenexa, Kans.) at 37°C under microaerophilic conditions (GasPak jars; BBL Microbiology Systems). Broth cultures of *H. pylori* were prepared by subculturing colonies from fresh agar plates into 2% (wt/vol) brucella broth (Difco Laboratories, Detroit, Mich.) containing 5% fetal bovine serum and incubating the broth cultures for about 24 h at 37°C in shake flasks (100 rpm) under microaerophilic conditions. Two of the four strains of *H. pylori* (ATCC 49503 and 43526) were grown under similar conditions in brucella broth supplemented with 5% fetal bovine serum and either 1% IsoVitaleX (Difco) or 0.25% yeast extract. These growth conditions provided the most consistent yields of viable cells from logarithmic-phase cultures for each of the four test strains of *H. pylori* used in these studies. Broth cultures showing inadequate or excessive growth of *H. pylori* after the 24-h incubation period were not used in killing studies. Frozen cultures of *H. pylori* were maintained at −70°C in brucella broth containing 20% (vol/vol) glycerol and 20% (vol/vol) fetal bovine serum. The absence of bacterial contaminants in *H. pylori* cultures was confirmed by subculture of broth cultures on blood agar plates and by monitoring the urease, oxidase, and catalase activities of the isolated colonies.

Materials. Monocaprylin ($C_{8:0}$ MG), monocaprin ($C_{10:0}$ MG), monolaurin ($C_{12:0}$ MG), caprylic acid ($C_{8:0}$ FFA), capric acid ($C_{10:0}$ FFA), and lauric acid ($C_{12:0}$ FFA) were obtained from Sigma Chemical Co. (St. Louis, Mo.). Other FFAs and MGs were purchased from Nu-Chek-Prep, Inc. (Elysian, Minn.). Metronidazole and tetracycline were obtained from Sigma.

Evaluation of bactericidal activity. Individual MGs and FFAs were evaluated

* Corresponding author. Mailing address: Bristol-Myers Squibb Co., Mead Johnson Research Center, 2400 W. Lloyd Expressway, Evansville, IN 47721. Phone: (812) 429-7472. Fax: (812) 429-5925.

TABLE 1. Bactericidal activity of medium-chain MGs and FFAs for *H. pylori*

Test MG or FFA	Concn (mM)	Change in no. ($\log_{10}$ CFU/ml) of viable *H. pylori* cells[a]			
		ATCC 43579	ATCC 43629	ATCC 49503	ATCC 43504
None (control)		−0.57 ± 0.92	−0.70 ± 0.69	0.15 ± 0.17	−0.05 ± 0.06
$C_{8:0}$ MG (monocaprylin)	1	−0.90 ± 1.23	−0.68 ± 0.97	0.01 ± 0.01	−0.04 ± 0.06
	5	−4.25 ± 0.21[b]	−4.15 ± 1.21[b]	−4.62 ± 0.41[b]	−5.11 ± 0.25[b]
$C_{10:0}$ MG (monocaprin)	1	−5.59 ± 0.78[b]	−5.68 ± 0.72[b]	−4.59 ± 0.42[b]	−5.11 ± 0.24[b]
	5	NT[c]	NT	NT	NT
$C_{12:0}$ MG (monolaurin)	1	−5.59 ± 0.78[b]	−5.42 ± 0.78[b]	−4.59 ± 0.41[b]	−5.14 ± 0.25[b]
	5	NT	NT	NT	NT
$C_{8:0}$ FFA (caprylic acid)	1	−0.66 ± 1.08	−0.89 ± 0.90	−0.05 ± 0.05	−0.08 ± 0.04
	5	−0.73 ± 1.23	−0.77 ± 1.10	−0.13 ± 0.18	−0.14 ± 0.04
$C_{10:0}$ FFA (capric acid)	1	−0.01 ± 0.01	−0.56 ± 0.74	−0.40 ± 0.56	−0.06 ± 0.02
	5	−0.56 ± 0.96	−0.53 ± 0.79	−0.08 ± 0.11	−0.04 ± 0.06
$C_{12:0}$ FFA (lauric acid)	1	−5.59 ± 0.78[b]	−5.42 ± 0.78[b]	−4.07 ± 1.24[b]	−5.11 ± 0.25[b]
	5	NT	NT	NT	NT

[a] Mean change (± standard deviation) in number of viable cells following a 1-h incubation of *H. pylori* with the indicated MG or FFA (pH 7). The initial inoculum for test bacteria was approximately 5×10^5 CFU/ml. Results of at least duplicate determinations are shown.
[b] Significantly different from control ($P < 0.05$).
[c] NT, not tested.

for in vitro bactericidal activity against *H. pylori* by monitoring the viability of test bacteria after a 1-h incubation in brucella broth containing a test MG or FFA. Briefly, approximately 5×10^5 washed bacterial cells were suspended in 1-ml aliquots of 2% brucella broth containing test MG or FFA samples and incubated for 1 h at 37°C under stationary conditions. The change in the number of viable bacterial cells was determined by standard plate counting procedures with either normal or chocolatized horse blood agar plates incubated for 3 to 5 days. Dilutions of test cultures for quantitative bacterial counts ranged from 10^{-1} to 10^{-4}, depending on the test MG or FFA, with a plating aliquot of 0.1 ml. The lower limit of detection was considered 100 CFU/ml. Carryover of MGs and FFAs could not be avoided by separation of MGs from cells via centrifugation prior to plating procedures for technical reasons relating to the pelleting of certain MGs with cells (possibly because of complexing with proteins, etc.) or "creaming" of other MGs to the top of the culture, which may have interfered with cell quantitation. Instead, carryover of test MGs was avoided by using dilutions of cultures for plating that would result in carryover levels below MIC ranges (e.g., dilutions of active MGs at the 1 mM level typically were 10^{-2} or greater, resulting in a carryover level of 10-fold or more below the MIC [0.01 mM]). Control tubes consisting of test bacterial cells incubated without MG or FFA were included in all experiments. Data were expressed as the log change in the number of viable cells ($\log_{10}$ CFU per milliliter) recovered after a 1-h incubation relative to the initial inoculum.

In certain assays the bactericidal activity of test MGs and FFAs was evaluated in nutritionally complete, liquid formula diets (Sustacal, Nutrament, and Ultracal; Mead Johnson and Company, Evansville, Ind.) diluted to half strength. MICs were determined by the standard agar dilution method (22a) with Mueller-Hinton agar supplemented with 5% horse blood.

Determination of frequency of resistance development. The frequency of in vitro resistance development was determined by the method described by Fernandes et al. (7, 13). Appropriate dilutions of overnight cultures of *H. pylori* were plated on horse blood agar plates containing from 2 to 10 times the estimated MIC of various MGs, FFAs, and antibiotics. Plates were incubated for 5 days at 37°C under microaerophilic conditions and then assessed for resistant colonies. The numbers of viable cells in the initial bacterial preparations were determined on blood agar plates without antimicrobial agents.

Statistical methods. Treatment effects on bacterial viability were analyzed by one-way analysis of variance and Tukey's multiple comparison procedure with log-transformed data (SAS/LAB, PC version 6.08). Levels of effectiveness against individual bacterial strains and in various diets were compared for each treatment by using multiple regression and analysis of variance. Differences were deemed statistically significant when the probability value was <0.05.

RESULTS

Inhibition of *H. pylori* by MGs and FFAs. The initial studies evaluated the in vitro bactericidal activity of various even-chain FFAs and MGs for several strains of *H. pylori* in a nutrient broth medium. Medium-chain MGs ($C_{8:0}$, $C_{10:0}$, and $C_{12:0}$) were consistently more bactericidal for *H. pylori* than medium-chain FFAs (Table 1). Four strains of *H. pylori* were found to be uniformly susceptible to 1 mM $C_{10:0}$ MG (monocaprin), 1 mM $C_{12:0}$ MG (monolaurin), and 1 mM $C_{12:0}$ FFA (lauric acid), showing 4-log-unit or greater reductions in numbers of viable cells ($P < 0.05$) after a 1-h incubation. Viable-cell numbers did not change in control cultures incubated without MG or FFA for 1 h under similar conditions. None of the *H. pylori* strains were affected by $C_{8:0}$ FFA (caprylic acid) or $C_{10:0}$ FFA (capric acid) at concentrations as high as 5 mM. Monocaprylin ($C_{8:0}$ MG) was less bactericidal for strains of *H. pylori*, showing no killing at 1 mM and approximately 3-log-unit reductions in viable-cell numbers at 5 mM ($P < 0.05$).

Comparison of saturated MGs and FFAs of various chain lengths for bactericidal activity against *H. pylori* showed 2-log-unit or greater reductions in the viability of *H. pylori* ($P < 0.05$) with MGs having carbon chain lengths ranging from C_9 to C_{14} ($C_{9:0}$, $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, $C_{13:0}$, and $C_{14:0}$) (Table 2). Lower levels of bactericidal activity were observed with $C_{15:0}$ and $C_{16:0}$ MGs. The bactericidal activity of a $C_{12:1}$ MG containing a single double bond in the 1 position was several thousandfold lower ($P < 0.05$) than that of the saturated form ($C_{12:0}$ MG), suggesting that the degree of saturation is important for bactericidal activity. In contrast to the bactericidal activity of MGs, lauric acid ($C_{12:0}$) was the only FFA among those tested (C_4 through C_{17}) that showed bactericidal activity against *H. pylori* ($P < 0.05$). Both $C_{10:0}$ MG and $C_{12:0}$ FFA were bactericidal for *H. pylori* under neutral pH and acidic conditions (Table 3). Incubation of control cultures at pH 4 or 5 showed no killing of

TABLE 2. Association of MG or FFA chain length with bactericidal activity for *H. pylori* ATCC 43629

MG or FFA chain length	Change in no. ($\log_{10}$ CFU/ml) of viable *H. pylori* cells after exposure to[a]:			
	FFA		MG	
	1 mM	5 mM	1 mM	5 mM
Control	−0.18 ± 0.17	−0.18 ± 0.17	−0.11 ± 0.11	−0.11 ± 0.11
$C_{4:0}$	−0.19 ± 0.23	−0.30 ± 0.20	NA[b]	NA
$C_{5:0}$	−0.21 ± 0.19	−0.23 ± 0.10	NA	NA
$C_{6:0}$	−0.17 ± 0.14	−0.19 ± 0.21	NA	NA
$C_{7:0}$	−0.16 ± 0.09	−0.19 ± 0.09	NA	NA
$C_{8:0}$	−0.89 ± 0.90	−0.77 ± 1.09	−0.68 ± 0.96	−4.15 ± 1.21[c]
$C_{9:0}$	−0.21 ± 0.11	−0.23 ± 0.11	−2.49 ± 0.64[c]	−4.74 ± 0.36[c]
$C_{10:0}$	−0.56 ± 0.74	−0.53 ± 0.79	−5.68 ± 0.72[c]	NT[d]
$C_{11:0}$	−0.43 ± 0.13	−0.48 ± 0.01	−4.78 ± 0.29[c]	−4.78 ± 0.29[c]
$C_{12:0}$	−5.42 ± 0.78[c]	NT	−5.42 ± 0.78[c]	NT
$C_{13:0}$	−0.50 ± 0.12	−0.60 ± 0.10	−4.78 ± 0.29[c]	−4.78 ± 0.30[c]
$C_{14:0}$	−0.20 ± 0.08	−0.71 ± 0.21	−4.07 ± 0.59[c]	−4.66 ± 0.25[c]
$C_{15:0}$	−0.43 ± 0.68	−0.54 ± 0.61	−1.91 ± 0.08[c]	−2.47 ± 0.78[c]
$C_{16:0}$	−0.34 ± 0.72	−0.36 ± 0.67	−1.41 ± 0.39	−1.48 ± 0.13
$C_{17:0}$	−0.43 ± 0.74	−0.45 ± 0.82	−0.28 ± 0.13	−0.18 ± 0.19
$C_{12:1}$	NA	NA	−1.69 ± 0.54	−2.54 ± 0.39[c]

[a] Mean change (± standard deviation) in number of viable cells following a 1-h incubation of *H. pylori* with the indicated MG or FFA (pH 7). The initial inoculum for test bacteria was approximately 5×10^5 CFU/mL. Results from duplicate determinations are shown.
[b] NA, not available.
[c] Significantly different from control ($P < 0.05$).
[d] NT, not tested.

H. pylori, while incubation at pH 3 caused a significant reduction in the number of viable *H. pylori* cells. The presence of 1 mM $C_{10:0}$ MG or $C_{12:0}$ FFA at pH 4, 5, or 7 led to 4-log-unit or greater reductions in the number of viable *H. pylori* cells compared with either a control culture at pH 7 without test MG or FFA or control cultures at the matched pH. While the viability of *H. pylori* in control cultures was reduced following incubation at pH 3, even greater killing of *H. pylori* was observed following incubation with $C_{10:0}$ MG or $C_{12:0}$ FFA at pH 3 (Table 3).

TABLE 3. Effect of pH on in vitro bactericidal activity of MG and FFA for *H. pylori* ATCC 43629

MG or FFA	pH	Change in no. ($\log_{10}$ CFU/ml) of viable *H. pylori* cells[a]
None (control)	7.0	5.65 ± 0.04
	5.0	0.21 ± 0.10
	4.0	−0.34 ± 0.51
	3.0	−2.64 ± 0.04[b]
$C_{10:0}$ MG (monocaprin)	7.0	−4.64 ± 0.04[b]
	5.0	−4.64 ± 0.04[b]
	4.0	−4.64 ± 0.04[b]
	3.0	−4.64 ± 0.04[b]
$C_{12:0}$ FFA (lauric acid)	7.0	−4.64 ± 0.04[b]
	5.0	−4.64 ± 0.04[b]
	4.0	−4.64 ± 0.04[b]
	3.0	−4.64 ± 0.04[b]

[a] Mean change (± standard deviation) in number of viable cells versus control at pH 7 following a 1-h incubation with 1 mM $C_{10:0}$ MG or $C_{12:0}$ FFA at the indicated pH. The initial inoculum for test bacteria was approximately 5×10^5 CFU/ml. Results from duplicate determinations are shown.
[b] Significantly different from pH 7 control ($P < 0.05$).

Evaluation of the bactericidal activity in high-protein liquid diets also showed greater reductions in *H. pylori* cell numbers after incubation with medium-chain MGs than after incubation with FFAs (Table 4). $C_{8:0}$, $C_{10:0}$, and $C_{12:0}$ MGs were bactericidal for *H. pylori* in liquid diets at 5 mM ($P < 0.05$). The activity of $C_{12:0}$ FFA, however, was considerably lower in liquid diets than in nutrient broth, requiring 20-fold-higher levels (20 mM) for 3-log-unit reductions in numbers of *H. pylori* cells. Neither $C_{8:0}$ FFA nor $C_{10:0}$ FFA was bactericidal for *H. pylori* at 20 mM in liquid diets. Interaction effects were also found for bactericidal activity by treatment and diet. $C_{8:0}$ MG was bactericidal for *H. pylori* at 5 mM in Nutrament ($P < 0.05$) but not in Sustacal or Ultracal. Conversely, $C_{12:0}$ FFA was bactericidal at 20 mM in Sustacal and Ultracal ($P < 0.05$) but not in Nutrament.

Development of resistance to MG or FFA. The relative frequency of spontaneous development of resistance to various MGs, $C_{12:0}$ FFA, tetracycline, and metronidazole among *H. pylori* strains was evaluated at 2, 5, and 10 times the MIC of each agent (Table 5). Preliminary studies found that the MICs of $C_{10:0}$ MG, $C_{12:0}$ MG, and $C_{12:0}$ FFA for *H. pylori* ranged from 0.12 to 0.25 mM. The MICs of tetracycline and metronidazole for the three test strains of *H. pylori* were approximately 0.0002 and 0.01 mM, respectively. These MICs are similar to those reported by Rubinstein et al. (26). In general, the frequency of spontaneous development of resistance among three strains of *H. pylori* was consistently higher for metronidazole and tetracycline than for MGs and lauric acid. While the pattern of susceptibility to metronidazole and tetracycline varied among the three test strains of *H. pylori*, the number of variants resistant to either metronidazole or tetracycline when tested at five times the MIC ranged from about 50 to $>1{,}000/10^8$ cells for each strain (frequency of resistance, 10^{-5} to 10^{-7}). As expected, the frequencies of spontaneous resistance mutations decreased with increasing concentrations of the test antibiotics. In contrast, no resistant variants of *H. pylori* were found when 10^8 cells were plated on media containing $C_{10:0}$ MG, $C_{12:0}$ MG, or $C_{12:0}$ FFA at concentrations as low as two times the MIC (frequency, $<10^{-8}$).

DISCUSSION

The in vitro antimicrobial properties of FFAs and fatty acid ester derivatives have been known for many years (2, 20, 23). The antibacterial activity associated with these compounds has been found with FFAs and MG derivatives of FFAs but not with di- or triacylglycerides (16). The results from our studies show for the first time that a variety of medium-chain MGs are bactericidal for *H. pylori*, the gram-negative bacterium associated with chronic superficial gastritis and peptic ulcer disease in humans. In contrast, other investigators have reported that a variety of gram-negative bacteria, including *Escherichia coli*, *Salmonella* spp., and *Proteus* spp., are not inhibited by FFAs or MGs (5, 15, 19). Our results indicate that the in vitro inhibitory activity of MGs against *H. pylori* is associated with both even- and odd-chain MG forms ranging in chain length from C_{10} to C_{14}, with MICs of $C_{10:0}$ MG and $C_{12:0}$ FFA, for example, ranging from 0.12 to 0.25 mM. Monolaurin, a saturated C_{12} MG, exhibited greater bactericidal activity than a $C_{12:1}$ MG containing a single double bond, indicating that saturation is important for bactericidal activity by medium-chain MGs. We also compared the antibacterial activities of odd- and even-chain FFAs ranging from $C_{4:0}$ to $C_{16:0}$ and found that lauric acid ($C_{12:0}$) was the only FFA that showed bactericidal activity against *H. pylori*. This finding is in contrast to results reported by others showing inhibition of gram-negative bacteria by a

TABLE 4. Bactericidal activity of MG and FFA for *H. pylori* ATCC 43629 in high-protein nutritional diets

Test MG or FFA	Concn (mM)	Change in no. ($\log_{10}$ CFU/ml) of viable *H. pylori* cells in liquid diet[a]		
		Sustacal	Nutrament	Ultracal
None (control)		−0.29 ± 0.11	0.02 ± 0.06	−0.18 ± 0.14
$C_{8:0}$ MG (monocaprylin)	5	−0.37 ± 0.51	−4.29 ± 0.08[b]	0.22 ± 1.55
	10	−3.66 ± 0.93[b]	−3.94 ± 0.41[b]	−3.64 ± 1.15[b]
$C_{10:0}$ MG (monocaprin)	5	−3.78 ± 0.41[b]	−4.00 ± 0.32[b]	−4.40 ± 0.07[b]
	10	−3.91 ± 0.45[b]	−4.14 ± 0.90[b]	−4.40 ± 0.07[b]
$C_{12:0}$ MG (monolaurin)	5	−3.41 ± 1.04[b]	−4.14 ± 0.52[b]	−3.04 ± 1.38[b]
	10	−3.77 ± 0.43[b]	−3.87 ± 0.12[b]	−4.40 ± 0.07[b]
$C_{8:0}$ FFA (caprylic acid)	10	0.51 ± 0.16	NT[c]	−0.20 ± 0.27
	20	0.19 ± 0.29	−0.15 ± 0.06	−0.36 ± 0.63
$C_{10:0}$ FFA (capric acid)	10	0.29 ± 0.12	NT	−0.05 ± 0.21
	20	0.29 ± 0.28	−0.07 ± 0.01	−0.06 ± 0.23
$C_{12:0}$ FFA (lauric acid)	10	−0.05 ± 0.49	NT	−1.05 ± 0.34
	20	−2.98 ± 0.50[b]	−0.05 ± 0.67	−4.01 ± 0.62[b]

[a] Mean change (± standard deviation) in number of viable cells following a 1-h incubation of *H. pylori* with MG or FFA in the indicated liquid diet. The initial inoculum for test bacteria was approximately 5×10^5 CFU/ml. Results of at least triplicate determinations are shown.
[b] Significantly different from control ($P < 0.05$).
[c] NT, not tested.

primarily short-chain FFA (27). While the test strains of *H. pylori* employed in our study exhibited similar levels of susceptibility to the antibacterial properties of MGs and FFAs, it should be recognized that our results were obtained with four laboratory strains of *H. pylori* as opposed to fresh clinical isolates.

Criteria to be considered during the evaluation of the antimicrobial activity of an agent against *H. pylori* should include not only the in vivo efficacy but also the ability of *H. pylori* strains to develop resistance to the agent. We evaluated the frequency of spontaneous emergence of resistance to MGs among strains of *H. pylori* for comparison with the development of resistance to metronidazole and tetracycline, two antimicrobial agents which are commonly included in current triple-therapy regimens used to treat *H. pylori* infections. Our results showed a much lower frequency of development of spontaneous resistance to $C_{10:0}$ MG, $C_{12:0}$ MG, and $C_{12:0}$ FFA than to metronidazole and tetracycline. The mechanism by which FFAs or MGs exert bacterial killing is thought to involve damage to the bacterial outer membrane leading to increased membrane fluidity and permeability (8, 9). Thompson et al. (29) found that the inhibitory effects of long-chain polyunsaturated fatty acids for *H. pylori* were associated with the incorporation of the unsaturated fatty acid into bacterial mem-

TABLE 5. Frequency of spontaneous development of resistance to MG, FFA, or antibiotics by *H. pylori*

Test agent	ATCC strain	MIC (mM)	Frequency of resistant mutants at concn of[a]:		
			2× MIC	5× MIC	10× MIC
$C_{10:0}$ MG (monocaprin)	43579	0.20	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
	43629	0.24	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
	49503	0.24	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
$C_{12:0}$ MG (monolaurin)	43579	0.18	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
	43629	0.18	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
	49503	0.18	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
$C_{12:0}$ FFA (lauric acid)	43579	0.12	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
	43629	0.25	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
	49503	0.12	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
Tetracycline	43579	0.0002	$>1 \times 10^{-5}$	$>1 \times 10^{-5}$	$<10^{-8}$
	43629	0.0002	$>1 \times 10^{-5}$	2.4×10^{-6}	$<10^{-8}$
	49503	0.0002	1.5×10^{-6}	$<10^{-8}$	$<10^{-8}$
Metronidazole	43579	0.02	$>1 \times 10^{-5}$	8.6×10^{-6}	5.1×10^{-6}
	43629	0.01	7.9×10^{-6}	$<10^{-8}$	$<10^{-8}$
	49503	0.01	$>1 \times 10^{-5}$	4.8×10^{-7}	$<10^{-8}$

[a] Number of spontaneous mutants found after incubation of *H. pylori* ($\sim 10^8$ CFU) on horse blood agar media containing the indicated concentration of MG, FFA, or antibiotic. Results are representative of data obtained from at least two individual experiments with each strain of *H. pylori.*

branes followed by development of abnormal cell forms and cell lysis. It is possible that medium-chain MGs or FFAs utilize a similar mechanism to inactivate bacteria, which might explain the low frequency of development of resistance to MGs in our study. Others have suggested that the bactericidal effects of polyunsaturated fatty acids are caused by toxic lipid peroxides generated from an oxidative process involving H_2O_2 and iron (19).

While numerous studies have confirmed the antibacterial activities of FFAs and MGs in vitro, evidence of in vivo benefits is still lacking. Several important questions regarding the efficacy of FFAs and MGs against *Helicobacter* infection of gastric tissues in vivo remain. Because FFAs and MGs are rapidly absorbed in the upper bowel (3), oral administration of MGs or FFAs may not be effective against enteric pathogens that inhabit the lower small intestine or colon. The amount of digestion and absorption of FFAs and MGs that occurs in the stomach, however, appears to be minimal (3, 6), suggesting that oral MGs may be effective against gastric infection with *H. pylori* in patients with gastritis or ulcer disease. It is not known whether orally administered MGs or FFAs will retain bactericidal activity against *H. pylori* in the presence of food and acid secretions in the lumen of the stomach. Previous studies have shown that the bactericidal activity of surface-active agents like MGs is markedly reduced in the presence of proteins, presumably because of the formation of lipid-protein complexes (10). Our results show that various medium-chain MGs and $C_{12:0}$ FFA (lauric acid) maintain bactericidal activity in the presence of high levels of protein in dietary supplements. However, higher levels of MGs, and especially of lauric acid, were needed to achieve the same level of bacterial killing. The effect of gastric secretions on the bactericidal activity of MGs and FFAs against *H. pylori* is also unknown. Our results further demonstrate that $C_{10:0}$ MG and $C_{12:0}$ FFA are bactericidal for *H. pylori* in vitro at both neutral and acidic pHs (4 to 7). The impact of the possible absorption of MGs by gastric tissues and gastric lipase activity on the availability of MGs or FFAs for inactivation of *H. pylori* is not known. Finally, it is not known whether FFAs or MGs are capable of penetrating the mucus layer to gain access and exert bactericidal effects on *H. pylori* that may be present either on epithelial surfaces or deep within gastric pits. These questions can be addressed only through careful evaluation of the effectiveness of MGs and FFAs against *Helicobacter* infections in either animal studies or human clinical trials.

ACKNOWLEDGMENTS

We thank Robert Talbott and Kristi Covalciuc for their technical contributions and Nancy West for her help in preparation of the manuscript.

REFERENCES

1. **Annibale, B., E. Rauws, and V. Savarino.** 1992. Long-term therapy of duodenal ulcer disease. Gastroenterol. Int. **5:**109–112.
2. **Bayliss, M.** 1936. Effect of the chemical constitution of soaps upon their germicidal properties. J. Bacteriol. **31:**489–504.
3. **Borgstrom, B., A. Dahlqvist, G. Lundh, and J. Sjovall.** 1957. Studies of intestinal digestion and absorption in the human. J. Clin. Invest. **36:**1521–1536.
4. **Christensen, A. H., R. P. H. Logan, L. A. Noach, and T. Gjørup.** 1994. Do clinicians accept the role of *Helicobacter pylori* in duodenal ulcer disease? A survey of European gastroenterologists and general practitioners. J. Intern. Med. **236:**501–505.
5. **Conley, A. J., and J. J. Kabara.** 1973. Antimicrobial action of esters of polyhydric alcohols. Antimicrob. Agents Chemother. **4:**501–506.
6. **Faber, J., R. Goldstein, O. Blondheim, H. Stankiewicz, A. Darwashi, J. A. Bar-Maor, A. Gorenstein, A. I. Eidelman, and S. Freier.** 1988. Absorption of medium-chain triglycerides in the stomach of the human infant. J. Pediatr. Gastroenterol. Nutr. **7:**189–195.
7. **Fernandes, P. B., C. W. Hanson, J. M. Stamm, C. Vojtko, N. L. Shipkowitz, and E. St. Martin.** 1987. The frequency of *in vitro* resistance development to fluoroquinolones and the use of a murine pyelonephritis model to demonstrate selection of resistance *in vivo*. J. Antimicrob. Chemother. **19:**449–465.
8. **Galbraith, H., and T. B. Miller.** 1973. Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts. J. Appl. Bacteriol. **36:**647–658.
9. **Galbraith, H., and T. B. Miller.** 1973. Effects of long chain fatty acids on bacterial respiration and amino acid uptake. J. Appl. Bacteriol. **36:**659–675.
10. **Glassman, H. N.** 1949. Surface active agents and their application in bacteriology. Bacteriol. Rev. **12:**105–148.
11. **Glupczynski, Y., A. Burette, E. de Koster, et al.** 1990. Metronidazole resistance in *Helicobacter pylori*. Lancet **335:**976–977. (Letter.)
12. **Graham, D. Y., G. M. Lew, H. M. Malaty, et al.** 1992. Factors influencing the eradication of *Helicobacter pylori* with triple therapy. Gastroenterology **102:**493–496.
13. **Hardy, D. J., C. W. Hanson, D. M. Hensey, J. M. Beyer, and P. B. Fernandes.** 1988. Susceptibility of *Campylobacter pylori* to macrolides and fluoroquinones. J. Antimicrob. Chemother. **22:**631–636.
14. **Hazell, S. L., and D. Y. Graham.** 1990. Unsaturated fatty acids and viability of *Helicobacter* (*Campylobacter*) *pylori*. J. Clin. Microbiol. **28:**1060–1061.
15. **Kabara, J. J.** 1978. Fatty acids and derivatives as antimicrobial agents. A review, p. 1–14. *In* J. J. Kabara (ed.), The pharmacological effects of lipids. American Oil Chemist Society, Champaign, Ill.
16. **Kabara, J. J., D. M. Swieczkowski, A. J. Conley, and J. P. Truant.** 1972. Fatty acids and derivatives as antimicrobial agents. Antimicrob. Agents Chemother. **2:**23–28.
17. **Kato, N., and I. Shibasaki.** 1975. Comparison of antimicrobial activities of fatty acids and their esters. J. Ferment. Technol. **53:**793–801.
18. **Kato, N., and I. Shibasaki.** 1976. Combined effect of citric and polyphosphoric acids on the antibacterial activity of monoglycerides. J. Antibact. Antifungal Agents (Jpn.) **4:**254–261.
19. **Knapp, H. R., and M. A. Melly.** 1986. Bactericidal effects of polyunsaturated fatty acids. J. Infect. Dis. **154:**84–94.
20. **Kodicek, E.** 1949. The effect of unsaturated fatty acids on gram-positive bacteria. Soc. Exp. Biol. Symp. **3:**217–232.
21. **Marshall, B. J.** 1993. Treatment strategies for *Helicobacter pylori* infection. Gastroenterol. Clin. North Am. **22:**183–198.
22. **Moss, S., and J. Calam.** 1992. *Helicobacter pylori* and peptic ulcers: the present position. Gut **33:**289–292.

22a. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.

23. **Nieman, C.** 1954. Influence of trace amounts of fatty acids on the growth of microorganisms. Bacteriol. Rev. **18:**147–163.
24. **Penston, J. G.** 1994. *Helicobacter pylori* eradication—understandable caution but no excuse for inertia. Aliment. Pharmacol. Ther. **8:**369–389. (Review.)
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. **Rubinstein, G., K. Dunkin, and A. J. Howard.** 1994. The susceptibility of *Helicobacter pylori* to 12 antimicrobial agents, omeprazole and bismuth salts. J. Antimicrob. Chemother. **34:**409–413.
27. **Sheu, C. W., and E. Freese.** 1973. Lipopolysaccharide layer protection of gram-negative bacteria against inhibition by long-chain fatty acids. J. Bacteriol. **115:**869–875.
28. **Shibasaki, I., and N. Kato.** 1978. Combined effects on antibacterial activity of fatty acids and their esters against gram-negative bacteria, p. 1–14. *In* J. J. Kabara (ed.), The pharmacological effects of lipids. American Oil Chemist Society, Champaign, Ill.
29. **Thompson, L., A. Cockayne, and R. C. Spiller.** 1994. Inhibitory effect of polyunsaturated fatty acids on the growth of *Helicobacter pylori*: a possible explanation of the effect of diet on peptic ulceration. Gut **35:**1557–1561.