ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1999, p. 374–376 Vol. 43, No. 2
0066-4804/99/$04.00+0

Mutations in 23S rRNA in *Helicobacter pylori* Conferring Resistance to Erythromycin Do Not Always Confer Resistance to Clarithromycin

M. ISABEL GARCÍA-ARATA,[1]* FERNANDO BAQUERO,[1] LUIS DE RAFAEL,[1] CARLOS MARTÍN DE ARGILA,[2] JAVIER P. GISBERT,[2] FERNANDO BERMEJO,[2] DANIEL BOIXEDA,[2] AND RAFAEL CANTÓN[1]

Departments of Microbiology[1] and Gastroenterology,[2] Hospital Ramón y Cajal, 28034 Madrid, Spain

Received 25 June 1998/Returned for modification 2 September 1998/Accepted 4 December 1998

Mutations conferring resistance to erythromycin or clarithromycin in *Helicobacter pylori* were studied. Mutation A2142G was consistently associated with clarithromycin MIC of >256 μg/ml, whereas mutants carrying A2143G had MICs ranging from ≤0.016 to >256 μg/ml, suggesting that additional factors account for the observed multiple levels of resistance to clarithromycin.

Helicobacter pylori is associated with several gastric conditions (1), and therapies including two antimicrobials (clarithromycin plus amoxicillin or metronidazole) plus a proton pump inhibitor are currently used (3, 6, 9).

In several bacterial species, mutations from adenine (A) to guanine (G) at positions 2058 or 2059 in domain V in the 23S rRNA gene in *Escherichia coli* confer resistance to several macrolides (20). Versalovic et al. (17) observed the association between A2059G or A2058G mutations and resistance to clarithromycin in *H. pylori* isolates from seven patients. Stone et al. (12, 13) reported the additional A2058C mutation in three clarithromycin resistant isolates. Taylor et al. (15) reassigned the former 2058 and 2059 as positions 2142 and 2143 based on their exact positions in the 23S rRNA gene in *H. pylori*. The aims of the present study were to analyze the association between macrolide resistance and point mutations at positions 2142 and 2143 in the gene coding for the 23S rRNA in *H. pylori* and to correlate clarithromycin MICs with these mutations.

Sixty-two erythromycin-resistant *H. pylori* isolates (MICs >256 μg/ml) plus 30 isolates susceptible to erythromycin (MICs ≤0.5 μg/ml) recovered from clinical samples from 89 patients were studied. Sensitivies to erythromycin and clarithromycin were determined by the E-test strips (AB Biodisk, Solna, Sweden) (5). Point mutations were detected by using PCR-restriction fragment length polymorphism (17). PCR products were used as a template in a cycle-sequencing reaction under the conditions described by Stone et al. (12).

Single point mutations (A2143G, A2142G, or A2142C) were detected in 100% (62 of 62 isolates) of the erythromycin-resistant isolates, but in none of the 30 erythromycin-susceptible isolates. Mutations at positions A2143G and A2142G were observed in 44 and 15 erythromycin-resistant isolates, respectively. In three isolates, no mutation by replacement of A with G was detected, at either position 2142 or 2143. The mutation A2142C was observed in three isolates by sequencing of PCR products. Such a change, A2142C, had been previously reported by Stone et al. (12), who found three isolates with this mutation among 41 clarithromycin-resistant isolates, and by Occhialini et al. (11), who reported that a single isolate was obtained from a patient in a survey carried out with seven patients.

The distribution of clarithromycin MICs is presented in Table 1. Clarithromycin MICs for isolates exhibiting the mutation A2143G ranged from ≤0.016 to ≥256 μg/ml. Two strains (6.6%) were inhibited by 0.016 μg/ml, 5 (16.6%) were inhibited by 0.5 to 2 μg/ml, 13 (43.3%) were inhibited by 4 to 64 μg/ml, and 10 (33.3%) were inhibited by ≥128 μg/ml. On the other hand, clarithromycin MICs for all isolates with the mutation at position 2142 (substitution of C or G) were >256 μg/ml.

Previously, differences in the clarithromycin MIC ranges for mutants with substitutions at the two positions have been suggested (13, 14, 18). Nevertheless, Szczebara et al. (14) included only eight clarithromycin-resistant isolates in their study. They found one isolate with A2142G mutation, which elicited a higher MIC than the remaining seven isolates, which all had the mutation at position 2143 and elicited MICs ranging from 2 to 32 μg/ml. However, they did not conclude that the mutation at position 2142 confers a higher resistance level. Stone et al. (13) found a statistically significant difference between the clarithromycin MICs for strains with mutations at the two positions when 32 μg/ml was chosen as a convenient concentration for discriminating the two populations. In the results presented here, such a statistically significant difference was also observed, in spite of a lower MIC (8 μg/ml) for discriminating the two populations (two-tailed Fisher's exact test, $P = 0.047$). Susceptible strains (with wild-type sequence) were consistently inhibited by concentrations of ≤0.032 μg/ml.

That strains with the same mutation, A2143G, elicit a wide range of MICs (from ≤0.016 to >256 μg/ml) may suggest the presence of mutations involving one or both 23S rRNA operons. Another possibility is an association with another resistance mechanism. Currently, other classic mechanisms of macrolide resistance, such as the presence of rRNA methylases or macrolide-efflux pumps, have not been detected in preliminary assays (7), but a closer view of the *H. pylori* complete genome sequence may provide further insight into this point (16). A number of putative ATP-binding cassettes and multidrug-efflux transporter genes have been identified in *H. pylori* based on sequence similarity (16). Indeed, Debets-Ossenkopp et al. (2) reported mutant strains with a low level of clarithromycin

* Corresponding author. Mailing address: Hospital Ramón y Cajal, Department of Microbiology, Carretera de Colmenar km 9.1, 28034 Madrid, Spain. Phone: (34) 91 336 83 30. Fax: (34) 91 336 88 09. E-mail: mloboa@meditex.es.

TABLE 1. Distribution of erythromycin and clarithromycin MICs for the mutations observed

Mutation	No. of strains	Erythromycin MIC (μg/ml)	Clarithromycin MIC (μg/ml)
A2142C	2	>256	>256
A2142G	10	>256	>256
A2143G	7	>256	>256
	1	>256	256
	2	>256	128
	3	>256	64
	4	>256	32
	3	>256	16
	2	>256	8
	1	>256	4
	2	>256	2
	2	>256	1
	1	>256	0.5
	2	>256	0.016
Wild-type sequence	1	0.5	<0.016
	2	0.25	<0.016
	1	0.125	0.023
	6	0.125	<0.016
	4	0.06	<0.016
	9	0.032	<0.016
	4	0.016	<0.016
	2	<0.016	<0.016

resistance and wild-type 23S rRNA, thus suggesting that *H. pylori* probably employs an additional mechanism to develop clarithromycin resistance.

Interestingly, when the strains in our study were selected by their resistance to erythromycin (as the representative generic antibiotic), at least seven strains exhibiting the A2143G mutation (first associated with clarithromycin resistance) and eliciting a clarithromycin MIC of ≤2 μg/ml were found. Therefore, from our study, erythromycin-resistant isolates are inferred to not necessarily be resistant to clarithromycin, even when they exhibit the A2143G mutation. To our knowledge, however, the presence of a specific mutation conferring resistance to erythromycin but not to clarithromycin has been reported only by Taylor et al. (15, 19), who studied five strains to characterize the 23S rRNA genes before and after the acquisition of clarithromycin resistance. In their study, four strains had the mutation A2142G and the fifth strain (strain B), with the A2143G mutation, elicited a clarithromycin MIC of 0.5 to 1 μg/ml as determined by agar dilution, a finding fully consistent with our results. Moreover, the clarithromycin MIC breakpoint chosen for *H. pylori* probably should be reconsidered. Most strains that produce a MIC above 0.12 μg/ml have a 23S rRNA mutation and cannot be considered fully susceptible, at least from the microbiological perspective. The risk of evolution of these strains towards ones manifesting higher levels of resistance during treatment remains to be evaluated. On clinical grounds, a concentration of 2 μg/ml has been chosen by most authors (11, 18, 21) to discriminate between susceptible and nonsusceptible strains, although the breakpoint is still an ill-defined issue. Recently, Wang and Taylor (19) categorized two strains for which the MICs of clarithromycin were 1 and 4 μg/ml as having an intermediate level of resistance.

Whether patients affected with strains with the A2143G mutation, which does not confer high-level clarithromycin resistance, can be safely treated with this drug or whether the strains will readily evolve to those with high-level clarithromycin resistance is at present unknown. Further studies to elucidate the effects of these mutations on the outcome of clarithromycin therapy are clearly warranted.

M.I. García-Arata received a grant from Abbott Laboratories, for which gratitude is hereby expressed. This work was supported in part by a project from Fondo de Investigaciones Sanitarias (exp. no. 97/0291).

REFERENCES

1. **Blaser, M. J.** 1997. Medical significance of *H. pylori. In* C. L. Clayton and H. L. T. Mobley (ed.), *Helicobacter pylori* protocols. Humana Press, New York, N.Y.
2. **Debets-Ossenkopp, Y. J., A. B. Brinkman, E. J. Kuipers, J. G. Kusters, and C. M. J. E. Vandenbroucke-Grauls.** 1997. Explaining the preferential A to G mutation in clarithromycin resistance in *Helicobacter pylori*, abstr. C-69. *In* Program and abstracts of the 37th International Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
3. **European *Helicobacter pylori* Study Group.** 1997. Current European concepts in the management of *Helicobacter pylori* infection. Gut **41:**8–13.
4. **Glupczynski, Y.** 1992. European multicentre study group on antibiotic susceptibility of *Helicobacter pylori*. Results of a multicentre European survey in 1991 of metronidazole resistance in *Helicobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis. **11:**777–781.
5. **Glupczynski, Y., M. Labbé, W. Hansen, F. Crokaert, and E. Yourassowsky.** 1991. Evaluation of the E-test for quantitative antimicrobial susceptibility testing of *Helicobacter pylori*. J. Clin. Microbiol. **29:**2072–2075.
6. **Graham, D. Y., W. A. de Boer, and G. N. J. Tytgat.** 1996. Choosing the best anti-*Helicobacter pylori* therapy: effect of antimicrobial resistance. Am. J. Gastroenterol. **91:**1072–1076.
7. **Hsieh, P. F., J. C. Yang, J. T. Lin, and J. T. Wang.** 1998. Molecular mechanisms of clarithromycin resistance in *Helicobacter pylori*. J. Formos. Med. Assoc. **97:**445–452.
8. **Hultén, K., A. Gibreel, O. Sköld, and L. Engstrand.** 1997. Macrolide resistance in *Helicobacter pylori*: mechanism and stability in strains from clarithromycin-treated patients. Antimicrob. Agents Chemother. **41:**2550–2553.
9. **Markham, A., and D. McTavish.** 1996. Clarithromycin and omeprazole as *Helicobacter pylori* eradication therapy in patients with *H. pylori*-associated gastric disorders. Drugs **51:**161–178.
10. **Neu, H. C.** 1991. The development of macrolides: clarithromycin in perspective. J. Antimicrob. Chemother. **27**(Suppl. A)**:**1–9.
11. **Occhialini, A., M. Urdaci, F. Doucet-Populaire, C. M. Bébéar, H. Lamouliatte, and F. Mégraud.** 1997. Macrolide resistance in *Helicobacter pylori*: rapid detection of point mutations and assays of macrolide binding to ribosomes. Antimicrob. Agents Chemother. **41:**2724–2728.
12. **Stone, G. G., D. Shortridge, R. K. Flamm, J. Versalovic, J. Beyer, K. Idler, L. Zulawinski, and S. K. Tanaka.** 1996. Identification of a 23S rRNA gene mutation in clarithromycin-resistant *Helicobacter pylori*. Helicobacter **1:**227–228.
13. **Stone, G. G., D. Shortridge, J. Versalovic, J. Beyer, R. K. Flamm, D. Y. Graham, A. T. Ghoneim, and S. K. Tanaka.** 1997. A PCR-oligonucleotide ligation assay to determine the prevalence of 23S rRNA gene mutations in clarithromycin-resistant *Helicobacter pylori*. Antimicrob. Agents Chemother. **41:**712–714.
14. **Szczebara, F., L. Dhaenens, P. Vincent, and M. O. Husson.** 1997. Evaluation of rapid molecular methods for detection of clarithromycin resistance in *Helicobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis. **16:**162–164.
15. **Taylor, D. E., Z. Ge, D. Purych, T. Lo, and K. Hiratsuka.** 1997. Cloning and sequence analysis of two copies of a 23S rRNA gene from *Helicobacter pylori* and association of clarithromycin resistance with 23S rRNA mutations. Antimicrob. Agents Chemother. **41:**2621–2628.
16. **Tomb, J.-F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539–547.
17. **Versalovic, J., D. Shortridge, K. Kibler, M. V. Griffy, J. Beyer, R. K. Flamm, S. K. Tanaka, D. Y. Graham, and M. F. Go.** 1996. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori*. Antimicrob. Agents Chemother. **40:**477–480.

18. **Versalovic, J., M. S. Osato, K. Spakovsky, M. P. Dore, R. Reddy, G. G. Stone, D. Shortridge, R. K. Flamm, S. K. Tanaka, and D. Y. Graham.** 1997. Point mutations in the 23S rRNA gene of *Helicobacter pylori* associated with different levels of clarithromycin resistance. J. Antimicrob. Chemother. **40:** 283–286.
19. **Wang, G., and D. E. Taylor.** 1998. Site-specific mutations in the 23S rRNA gene of *Helicobacter pylori* confer two types of resistance to macrolide-lincosamide-streptogramin B antibiotics. Antimicrob. Agents Chemother. **42:**1952–1958.
20. **Weisblum, B.** 1995. Erythromycin resistance by ribosome modification. Antimicrob. Agents Chemother. **39:**577–585.
21. **Xia, H.-X., M. Buckley, C. T. Keane, and C. A. O'Morain.** 1997. Clarithromycin resistance in *Helicobacter pylori*: prevalence in untreated dyspeptic patients and stability in vitro. J. Antimicrob. Chemother. **40:**283–286.