ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1993, p. 1203–1205
0066-4804/93/051203-03$02.00/0

Vol. 37, No. 5

In Vitro Activity of Azithromycin against Bacterial Enteric Pathogens

MANUEL E. GORDILLO,[1]† KAVINDRA V. SINGH,[1] AND BARBARA E. MURRAY[1,2]*

Center for Infectious Diseases, Department of Internal Medicine,[1] and Department of Microbiology and Molecular Genetics,[2] The University of Texas Medical School at Houston, Houston, Texas 77030

Received 14 December 1992/Accepted 10 March 1993

The in vitro activity of azithromycin against enteric bacterial pathogens was determined by agar dilution. Azithromycin was highly active against *Campylobacter* spp. (MIC for 90% of strains tested [MIC_{90}] = 0.125 μg/ml) and against enterotoxigenic, enterohemorrhagic, enteroinvasive, and enteropathogenic *Escherichia coli* (MIC_{90} = 2 μg/ml), *Shigella* spp. (MIC_{90} = 1 μg/ml), and *Salmonella* spp. (MIC_{90} = 4 μg/ml), including *Salmonella typhi* (MIC_{90} = 1 μg/ml). On the basis of the in vitro activity of the drug against these organisms, clinical studies of azithromycin in enteric diseases should be considered; the high intracellular concentrations achieved by azithromycin may be particularly relevant for organisms like *S. typhi*, *Campylobacter* spp., and *Shigella* spp. which typically invade cells as part of their infectious process.

Infectious diarrhea is a leading cause of death in developing countries, where it has its greatest impact in children less than 5 years old. Estimates of fatalities in this population exceed 4.5 million per year (33). In highly industrialized countries, infectious diarrhea also causes significant morbidity among children (leading to many hours lost from work among parents) and among travelers to less developed countries (13, 16). In addition, infectious diarrhea has been shown to be a significant contributor to mortality among the elderly, especially those who are institutionalized (22). Although oral rehydration is the mainstay of treatment of infectious diarrhea, in selected situations, such as with severe systemic illness caused by invasive bacterial organisms and during outbreaks, antibiotics can play a role in curtailing morbidity, reducing mortality, and decreasing the shedding and subsequent spread of organisms (31).

One of the major limitations to successful antimicrobial therapy of bacterial enteric pathogens has been the progressive emergence of resistance to these agents, particularly in developing countries (25). Indeed, antimicrobial resistance was recognized as an important clinical problem in the 1950s when isolates of *Shigella dysenteriae* were observed to have acquired resistance to all of the then available agents (sulfonamide, tetracycline, chloramphenicol, and streptomycin). Similarly, the appearance of resistance to ampicillin, trimethoprim, and trimethoprim-sulfamethoxazole followed shortly after these agents were introduced into clinical use (25). Currently, in some parts of the world, more than 80% of some species of *Shigella* and *Salmonella* are resistant to trimethoprim-sulfamethoxazole and to most or all of the other useful oral agents (8, 10, 17, 21). *Campylobacter* spp., another important cause of more severe diarrhea, are characteristically resistant to conventional antimicrobial agents such as ampicillin and trimethoprim-sulfamethoxazole. More recently, acquired resistance to erythromycin and fluoroquinolones has been reported (1, 32, 34, 35); erythromycin-resistant campylobacters have shown cross-resistance to other macrolides (34).

With the marked increase in antibiotic resistance among enteric bacterial pathogens, it has become imperative to find alternative effective antimicrobial agents. Among the oral antimicrobial agents, the fluoroquinolones and oral broad-spectrum cephalosporins are the only groups whose efficacy against diarrheagenic species of the family *Enterobacteriaceae* has not yet been compromised by acquired resistance. However, the former agents are not yet recommended for use in pediatric patients because of articular cartilage damage caused by these drugs in young animals, and the latter agents are quite expensive. There is also concern that extensive use of oral broad-spectrum cephalosporins in the outpatient setting will select for derivatives of the widespread TEM-like β-lactamases that can hydrolyze broad-spectrum cephalosporins, as has been observed in the hospital setting (26). Erythromycin is an old antibiotic which has been used to a limited degree to decrease colonization and to prevent infections caused by enteric gram-negative bacilli (2, 4). Although members of the *Enterobacteriaceae* are typically resistant to erythromycin (MIC, 2 to 256 μg/ml [2, 4]), intestinal drug levels are higher than these MICs and oral administration of erythromycin results in a marked decrease in intestinal *Enterobacteriaceae* colonization (2–4). Roxithromycin has also been shown to decrease counts of gram-negative enteric bacilli (29). Use of erythromycin is limited by frequent gastrointestinal side effects and, because of the high MICs, would presumably not be useful for treating infections caused by those members of the *Enterobacteriaceae* which invade beyond the intestinal lumen (e.g., *Shigella* and *Salmonella* spp.).

Azithromycin, a new azalide recently approved for marketing in the United States, has good in vitro activity against *Campylobacter* spp. and has been shown to inhibit a limited number of gram-negative enteric pathogens. To further explore the possible usefulness of azithromycin for bacterial diarrhea, we determined the in vitro activity of azithromycin against bacterial enteric pathogens and compared these results with those obtained with erythromycin, a related macrolide commonly used to treat severe *Campylobacter* diarrhea.

The bacterial isolates were from the culture collection at the University of Texas Health Science Center at Houston; the organisms were isolated from patients in Mexico, South

* Corresponding author.
† Present address: Manasota Infectious Disease Consultants, Sarasota, FL 34239.

TABLE 1. In vitro activity of erythromycin and azithromycin against enteric pathogens

Organism (no. of isolates)[a]	MIC (μg/ml) of[b]:					
	Erythromycin			Azithromycin		
	50%	90%	Range	50%	90%	Range
Campylobacter spp. (19[c]) at:						
pH 7.4	1	2	0.25–2	0.06	0.125	0.03–0.125
pH 8.0	0.5	1.0	0.25–2	0.06	0.125	0.06–0.125
ETEC (35)	16	32	2–256	0.5	2	0.5–8
EIEC (15)	8	16	8–16	0.5	1	0.25–1
EPEC (11)	16	32	8–32	1	2	0.5–2
EHEC (14)	8	16	8–16	1	1	0.5–2
Salmonella spp. (20)	32	64	16–64	1	4	1–4
S. typhi (16)	16	16	8–256	0.5	1	0.25–2
Shigella spp. (20)	16	16	4–64	1	1	0.5–1

[a] Abbreviations: ETEC, enterotoxigenic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; EHEC, enterohemorrhagic *E. coli*.
[b] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[c] Includes 17 *C. jejuni* isolates and 2 *C. coli* isolates.

America, Southeast Asia, and the United States over the past decade and were identified to the species level by routine biochemical tests. For presumptive identification of enterotoxigenic *Escherichia coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli*, and enterohemorrhagic *E. coli*, previously described gene probes were used (9, 23, 27, 36).

Azithromycin was supplied by Pfizer Inc., New York, N.Y., and erythromycin was obtained from U.S. Biochemical Corp., Cleveland, Ohio. MICs were determined by agar dilution using serial twofold dilutions in Campy blood agar (Adams Scientific Inc., Sulphur, La.) at pH 7.4 and 8.0 for *Campylobacter* spp. and in Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) for all other isolates. Different pHs were evaluated because previous studies have shown marked variability in MICs of azithromycin against enteric gram-negative bacilli in broth (7). An effect of pH on activity might have clinical relevance for diarrheal disease since the lower intestinal contents are typically alkaline. Bacteria were inoculated from an overnight culture and adjusted to a 0.5 McFarland standard (28). A 1:10 dilution was made in Mueller-Hinton broth, and an inoculum replicating device (Steers) was used to apply a final inoculum of 10^4 CFU per spot, as per standards of the National Committee for Clinical Laboratory Standards (28). Inoculated plates were incubated at 37°C for 18 h; plates inoculated with *Campylobacter* cultures were incubated at 42°C in a Campy Pak (Becton Dickinson and Co., Cockeysville, Md.) for 48 h. Control organisms, including *Staphylococcus aureus* 29213, *Streptococcus* (*Enterococcus*) *faecalis* 29212, and *E. coli* 25922, were included with each set of isolates tested.

The MICs for 50% of strains tested (MIC_{50}s), MIC_{90}s, and MIC ranges are shown in Table 1. The greatest activity of azithromycin occurred against *Campylobacter* spp., with 50% of isolates inhibited by 0.05 μg/ml and 90% inhibited by 0.125 μg/ml; results were the same at both pHs tested. In comparison, erythromycin inhibited 50% of isolates at 1 μg/ml and 90% of isolates at 2 μg/ml at pH 7.4 and was 1 dilution more active at pH 8. These results are consistent with data previously reported for both drugs (19, 20) but lower than the MICs found by Taylor and Chang (34). The latter investigators, however, did not specify the pH conditions of their test and specifically studied a large proportion of erythromycin-resistant isolates. We did not encounter any erythromycin-resistant isolates among our campylobacters, but other organisms that are erythromycin resistant, including the campylobacters tested by Taylor and Chang, are cross resistant to azithromycin (14, 30, 34).

For enterotoxigenic, enteroinvasive, enterohemorrhagic, and enteropathogenic *E. coli*, azithromycin was 8 to 10 times more active than erythromycin, in keeping with the results reported by Jones et al. for enterotoxigenic and enteroinvasive *E. coli* (18). Ninety percent of *Salmonella* spp. and *Salmonella typhi* were inhibited by 4 and 1 μg of azithromycin per ml, respectively. In the case of *S. typhi*, these results agree with those of a previous report (24) which also suggested that azithromycin might be a useful alternative in selected cases of typhoid fever, since very high concentrations of this antibiotic are achieved in phagocytic cells and tissues (15). The efficacies of azithromycin in mice infected with *Salmonella typhimurium* and as treatment of typhoid fever in humans also support the possibility that azithromycin has value as a therapeutic agent for enteric infections (11, 12). The MIC_{90} of azithromycin of 1 μg/ml for *Shigella* spp. confirms previous data for these organisms (18, 30).

For all the gram-negative bacilli we tested, the MICs of erythromycin were high ($MIC_{90} \geq 16$ μg/ml), as would be expected from previously reported data for *Enterobacteriaceae* (37). The MIC was ≥500 μg/ml for all organisms, and thus these organisms are likely displaying the intrinsic resistance typical of enteric gram-negative bacilli to erythromycin (6). High-level resistance to erythromycin has been reported among fecal isolates of *Enterobacteriaceae* from cancer patients in a setting where erythromycin was widely used to decrease gram-negative intestinal colonization (3). This was due at least in part to a gene almost identical to *ermAM* of gram-positive organisms (5). The erythromycin MICs for these highly resistant organisms were >1,000 μg/ml. Such organisms would likely be resistant to azithromycin, as are erythromycin-resistant gram-positive cocci and campylobacters (14, 30), but highly resistant isolates were not encountered in our collection.

In this study, we have shown excellent in vitro activity of azithromycin against the most common bacterial enteric pathogens. The presence of high intracellular concentrations suggests that this compound may have advantages over standard therapies for invasive enteric organisms. The fact that no organisms with high-level erythromycin resistance (which would likely be cross resistant to azithromycin) were encountered is encouraging since erythromycin has been widely used for many decades in the outpatient setting. This does not mean that azithromycin resistance will not emerge, but it at least shows that resistant enteric pathogens are not common at the current time. Azithromycin represents a particularly attractive potential alternative in the pediatric population, since isolates resistant to antimicrobial agents usually recommended for enteric infections are increasingly common and since the fluoroquinolones are not usually recommended for this age group. Further studies to define the role of azithromycin in the management of bacterial diarrhea may be warranted.

This study was supported by a grant from Pfizer Inc.

REFERENCES

1. **Alder-Mosca, H., J. Luthy-Hottenstein, G. M. Lucchini, A. Burnens, and M. Altwegg.** 1991. Development of resistance to quinolones in five patients wtih campylobacteriosis treated with norfloxacin or ciprofloxacin. Eur. J. Clin. Microbiol. **10:**953–957.
2. **Andremont, A., P. Raibaud, and C. Tancrede.** 1983. Effect of

erythromycin on microbial antagonisms: a study in gnotobiotic mice associated with a human fecal flora. J. Infect. Dis. **148:** 579–587.

3. **Andremont, A., H. Sancho-Garnier, and C. Tancrede.** 1986. Epidemiology of intestinal colonization by members of the family *Enterobacteriaceae* highly resistant to erythromycin in a hematology-oncology unit. Antimicrob. Agents Chemother. **29:** 1104–1107.
4. **Andremont, A., and C. Tancrede.** 1981. Reduction of the aerobic gram-negative bacterial flora of the gastro-intestinal tract and prevention of traveller's diarrhea using oral erythromycin. Ann. Microbiol. **132B:**419–427.
5. **Arthur, M., A. Andremont, and P. Courvalin.** 1987. Distribution of erythromycin esterase and rRNA methylase genes in members of the family *Enterobacteriaceae* highly resistant to erythromycin. Antimicrob. Agents Chemother. **31:**404–409.
6. **Arthur, M., and P. Courvalin.** 1986. Contribution of two different mechanisms to erythromycin resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **30:**694–700.
7. **Barry, A. L., and P. C. Fuchs.** 1991. In-vitro potency of azithromycin against gram-negative bacilli is method-dependent. J. Antimicrob. Chemother. **28:**607–608. (Letter.)
8. **Bennish, M. L., M. A. Salam, M. A. Hossain, J. Myaux, E. H. Khan, J. Chakraborty, F. Henry, and C. Ronsmans.** 1992. Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983–1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. Clin. Infect. Dis. **14:**1055–1060.
9. **Bower, J. R., B. L. Congeal, T. G. Cleary, R. T. Stone, A. Wanger, B. E. Murray, J. J. Mathewson, and L. K. Pickering.** 1989. *Escherichia coli* 0114:nonmotile as a pathogen in an outbreak of severe diarrhea associated with a day care center. J. Infect. Dis. **160:**243–247.
10. **Bratoeva, M. P., J. F. John, and N. L. Barg.** 1992. Molecular epidemiology of trimethoprim-resistant *Shigella boydii* serotype 2 strains from Bulgaria. J. Clin. Microbiol. **30:**1428–1431.
11. **Butler, T.** 1991. Treatment of experimental *Salmonella typhimurium* infection in mice with azithromycin, abstr. A-65, p. 11. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
12. **Butler, T., C. Palomino, R. B. Johnson, and S. J. Hopkins.** 1992. Efficacy of azithromycin for treatment of typhoid fever, abstr. 1579, p. 374. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.
13. **DuPont, H. L., and B. S. Ribner.** 1986. Infectious gastroenteritis, p. 495–508. *In* J. V. Bennett and P. S. Brachman (ed.), Hospital infections. Little, Brown & Co., Boston.
14. **Fernandes, P. B., W. R. Baker, L. A. Freiberg, D. J. Hardy, and E. J. McDonald.** 1989. New macrolides active against *Streptococcus pyogenes* with inducible or constitutive type of macrolide-lincosamide-streptogramin B resistance. Antimicrob. Agents Chemother. **33:**78–81.
15. **Girard, A. E., D. Girard, and J. A. Retsema.** 1990. Correlation of the extravascular pharmacokinetics of azithromycin with in-vivo efficacy in models of localized infection. J. Antimicrob. Chemother. **25**(Suppl. A)**:**61–71.
16. **Gorbach, S. L.** 1986. Travelers' diarrhea, p. 179–190. *In* S. L. Gorbach (ed.), Infectious diarrhea. Blackwell Scientific Publications, Boston.
17. **Hyath, K. C., A. L. Bourgeois, and B. R. Merrel.** 1991. Diarrheal disease during Operation Desert Shield. N. Engl. J. Med. **325:**1423–1428.
18. **Jones, K., D. Felmingham, and G. Ridgway.** 1988. *In vitro* activity of azithromycin (CP-62,993), a novel macrolide, against enteric pathogens. Drugs Exp. Clin. Res. **XIV:**613–615.
19. **Karmali, M. A., S. De Grandis, and P. C. Fleming.** 1981. Antimicrobial susceptibility of *Campylobacter jejuni* with special reference to resistance patterns of Canadian isolates. Antimicrob. Agents Chemother. **19:**593–597.
20. **Kitzis, M. D., F. W. Goldstein, M. Miegi, and J. F. Acar.** 1990. In-vitro activity of azithromycin against various gram-negative bacilli and anaerobic bacteria. J. Antimicrob. Chemother. **25**(Suppl. A)**:**15–18.
21. **Lepage, P., J. Bogaerts, C. Van Goethem, D. G. Hitimana, and F. Nsengumuremyi.** 1990. Multiresistant *Salmonella typhimurium* systemic infection in Rwanda. Clinical features and treatment with cefotaxime. J. Antimicrob. Chemother. **26**(Suppl. A)**:**53–57.
22. **Lew, J. F., R. I. Glass, R. E. Gangarosa, I. P. Cohen, C. Bern, and C. L. Moe.** 1991. Diarrheal deaths in the United States, 1979 through 1987. JAMA **265:**3280–3283.
23. **Lopez, E. L., M. Diaz, S. Devoto, S. Grinstein, M. Woloj, B. E. Murray, E. Rubeglio, F. Mendilaharzu, M. Turco, M. Vasquez, L. K. Pickering, and T. G. Cleary.** 1991. Evidence of infection with organisms producing Shiga-like toxins in household contacts of children with the hemolytic uremic syndrome. Pediatr. Infect. Dis. J. **10:**20–24.
24. **Metchock, B.** 1990. In-vitro activity of azithromycin compared with other macrolides and oral antibiotics against *Salmonella typhi*. J. Antimicrob. Chemother. **24**(Suppl. A)**:**29–31.
25. **Murray, B. E.** 1986. Resistance of *Shigella*, *Salmonella*, and other selected enteric pathogens to antimicrobial agents. Rev. Infect. Dis. **8**(Suppl. 2)**:**S172–S181.
26. **Murray, B. E.** 1991. New aspects of antimicrobial resistance and the resulting therapeutic dilemmas. J. Infect. Dis. **163:**1185–1194.
27. **Murray, B. E., J. J. Mathewson, H. L. DuPont, and W. E. Hill.** 1987. Utility of oligodeoxyribonucleotide probes for detecting enterotoxigenic *Escherichia coli*. J. Infect. Dis. **155:**809–811.
28. **National Committee for Clinical Laboratory Standards.** 1988. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Second edition, tentative standard. M7-T2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
29. **Pecquet, S., E. Chachaty, C. Tancrede, and A. Andremont.** 1991. Effects of roxithromycin on fecal bacteria in human volunteers and resistance to colonization in gnotobiotic mice. Antimicrob. Agents Chemother. **35:**548–552.
30. **Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, L. Brennan, and R. Mason.** 1987. Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. Antimicrob. Agents Chemother. **31:**1939–1947.
31. **Sack, R. B.** 1986. The treatment of diarrhea with antibiotics, p. 227–236. *In* S. L. Gorbach (ed.), Infectious diarrhea. Blackwell Scientific Publications, Boston.
32. **Segreti, J., T. D. Gootz, L. J. Goodman, G. W. Parkhurst, J. P. Quinn, B. A. Martin, and G. M. Trenholme.** 1992. High-level quinolone resistance in clinical isolates of *Campylobacter jejuni*. J. Clin. Infect. Dis. **165:**667–670.
33. **Snyder, J. D., and M. H. Merson.** 1982. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. Bull. W.H.O. **60:**605–613.
34. **Taylor, D. E., and N. Chang.** 1991. In vitro susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* to azithromycin and erythromycin. Antimicrob. Agents Chemother. **35:**1917–1918.
35. **Taylor, D. N., M. J. Blaser, P. Echeverria, C. Pitarangsi, L. Bodhidatta, and W.-L. L. Wang.** 1987. Erythromycin-resistant *Campylobacter* infections in Thailand. Antimicrob. Agents Chemother. **31:**438–442.
36. **Wanger, A. R., B. E. Murray, P. Echeverria, J. J. Mathewson, and H. L. DuPont.** 1988. Enteroinvasive *Escherichia coli* in travelers with diarrhea. J. Infect. Dis. **158:**640–642.
37. **Washington, J. A., and W. R. Wilson.** 1985. Erythromycin: a microbial and clinical perspective after 30 years of clinical use. Mayo Clin. Proc. **60:**271.