ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1994, p. 1186–1189
0066-4804/94/$04.00+0

Vol. 38, No. 5

Rifampin for Therapy of Experimental Pneumococcal Meningitis in Rabbits

ROLAND NAU,† KEITH KAYE, MEENA SACHDEVA, ERIC R. SANDE, AND MARTIN G. TÄUBER*

Infectious Diseases Laboratory, San Francisco General Hospital, San Francisco, California 94110, and Department of Medicine, University of California San Francisco, San Francisco, California 94143-0811

Received 1 November 1993/Returned for modification 18 January 1994/Accepted 28 February 1994

Rifampin at a maximally effective dose was less active than ceftriaxone (both drugs at 10 mg/kg of body weight · h) in a rabbit model of pneumococcal meningitis ($\Delta\log_{10}$ CFU/ml · h, -0.40 ± 0.13 versus -0.77 ± 0.18; $P < 0.01$). The bactericidal activity of rifampin decreased at concentrations in cerebrospinal fluid greater than those that are clinically achievable, and use of rifampin in combination with ofloxacin had no synergistic or additive effect.

Pneumococci resistant to multiple antibiotics are an increasing challenge (13). At present, more than 10% of the pneumococci isolated in Spain, South Africa, Mexico, Poland, and Kenya are not susceptible to penicillin G (11). Penicillin resistance can substantially complicate the therapy of pneumococcal meningitis. In a study from Spain, 15 of 66 *Streptococcus pneumoniae* strains isolated from patients with meningitis were only moderately susceptible or resistant to penicillin G, and 7 of these strains were also resistant to chloramphenicol (22). Furthermore, resistance to penicillin is regularly associated with reduced susceptibilities to other β-lactams, and failures of cefotaxime and ceftriaxone for the treatment of meningitis caused by penicillin-resistant *S. pneumoniae* have been observed (1, 2, 8). Rifampin has been used successfully as an adjunct in the treatment of central nervous system infections caused by *S. pneumoniae* and staphylococci (15, 17). This prompted us to evaluate rifampin, which has low MICs for most strains of penicillin-resistant *S. pneumoniae* (11, 22), in a rabbit model of experimental pneumococcal meningitis (3). We also examined whether increased bactericidal activity could be achieved by the combination of rifampin-ofloxacin. Rifampin-quinolone combinations have been used to treat other infections (6), and ofloxacin is one of the more active clinically available quinolones against pneumococci on the basis of the ratio of its MIC to achievable levels in serum. Ceftriaxone was used as the comparison drug in the studies.

In vitro studies. All studies were performed with a penicillin-susceptible *S. pneumoniae* type 3 strain (MIC and MBC of penicillin G, 0.03 μg/ml) originally isolated from a patient with meningitis (20). MICs and MBCs were determined by a standard broth dilution method by using Todd-Hewitt broth (THB; Difco Laboratories, Detroit, Mich.) and an inoculum of 10^5 CFU/ml. The MIC was defined as the lowest concentration of an antibiotic that inhibited visible growth after 24 h of incubation at 37°C in room air with 5% CO_2. The MBC was defined as the lowest antibiotic concentration that reduced the initial inoculum by >99.9%. The MIC and MBCs for the experimental strain of *S. pneumoniae* were, respectively, 0.008 and 0.06 μg/ml for rifampin, 0.5 and 1 μg/ml for ofloxacin, and 0.03 and 0.06 μg/ml for ceftriaxone. The in vitro bactericidal activity of rifampin was examined by time-kill studies in tubes containing 4 ml of THB and rifampin at concentrations of 1 to 200 times the MBC (0.06 to 12 μg/ml) ($n = 4$). Initial titers were 6×10^5 to 6×10^6 CFU/ml, and changes in bacterial titers were examined by quantitative subculturing every 2 h for a total of 6 h. The bactericidal activities produced by rifampin were very similar at all concentrations examined, with a decrease of approximately $\log_{10}$ 0.2 CFU/ml · h, and we found no evidence of a dose-response. The mean ± standard deviation bactericidal activities of rifampin in vitro in time-kill experiments over 6 h at rifampin concentrations of 0, 0.06, 0.6, 1.2, 3.6, and 12.0 μg/ml were 0.315 ± 0.136, −0.238 ± 0.136, −0.141 ± 0.063, −0.174 ± 0.113, −0.230 ± 0.204, and −0.218 ± 0.117, respectively. ($n = 4$ for all determinations). There was no significant difference in the rate of bactericidal activity at different concentrations of rifampin (by analysis of variance, $P = 0.81$; $P < 0.05$ for active drug versus control).

Rabbit model. After intramuscular anesthesia with ketamine (30 mg/kg of body weight), xylazine (15 mg/kg), and acepromazine (3 mg/kg), New Zealand White rabbits (weight, 2 to 3 kg) were inoculated intracisternally with 10^6 CFU of the experimental pneumococcus. The inoculum was prepared as follows. Overnight cultures on blood agar plates of cerebrospinal fluid (CSF) from rabbits infected with the organism were suspended in saline and were kept at −70°C. These stock cultures were then thawed and diluted in saline to the desired concentration and were injected into the rabbits. After infection, animals were returned to their cages. Eighteen hours later, when animals showed signs of meningitis, they were anesthetized by the intravenous administration of urethane (2 g/kg). CSF (0.3 ml) was obtained by puncturing the cisterna cerebellomedullaris for an initial assessment of bacterial titers. Subsequently, antibiotic therapy with rifampin (Rifadin; Marion Merrel Dow, Kansas City, Mo.) at 5 mg/kg · h ($n = 12$), 10 mg/kg · h ($n = 14$), or 20 mg/kg · h ($n = 14$); rifampin at 5 mg/kg · h and ofloxacin (Floxin; Johnson & Johnson, Arlington, Tex.) at 10 mg/kg · h ($n = 10$); or rifampin at 10 mg/kg · h and ofloxacin at 40 mg/kg · h ($n = 5$) was administered intravenously continuously for 7 h. A group receiving ceftriaxone (Roche Laboratories, Nutley, N.J.) at 10 mg/kg · h ($n = 8$) and an untreated group receiving 0.9% saline ($n = 12$) served for comparison. The high doses of ofloxacin and rifampin, which produced concentrations in excess of those observed in humans, were chosen to determine the dose dependency of bacterial killing. A loading dose, the size of which was previ-

* Corresponding author. Mailing address: Division of Infectious Diseases, University of California, San Francisco, Box 0811, 3rd and Parnassus Avenues, San Francisco, CA 94143. Phone: (415) 206-8844. Fax: (415) 206-6015.

† Present address: Department of Neurology, Georg-August-University, 37075 Göttingen, Germany.

TABLE 1. Concentrations of study drugs in serum and CSF and bactericidal activity in CSF of rabbits with experimental pneumococcal meningitis

Drug (dose [mg/kg · h])	No. of rabbits	Concn (μg/ml)				Concn in CSF/serum ratio (7 h)	$\Delta\log_{10}$ CFU/ml · h
		Serum, 1 h	CSF, 1 h	Serum, 7 h	CSF, 7 h		
Saline	12	NA[a]	NA	NA	NA	NA	+0.22 ± 0.07[b]
Rifampin (5)	12	9.5 ± 2.2	0.2 ± 0.1	9.7 ± 2.6	2.2 ± 1.3	0.22	−0.24 ± 0.13
Rifampin (10)	14	23.1 ± 9.7	0.8 ± 0.3	25.8 ± 9.7	4.6 ± 1.7	0.18	−0.40 ± 0.13[c]
Rifampin (20)	12	66.0 ± 26.5	1.9 ± 0.8	70.8 ± 26.3	12.9 ± 3.3	0.18	−0.17 ± 0.14
Rifampin (5) + ofloxacin (10)	10	ND[d]	ND	ND	ND	ND	−0.12 ± 0.12[e]
Rifampin (10) + ofloxacin (40)	5	ND	ND	ND	ND	ND	−0.49 ± 0.12[f]
Ofloxacin (10)	14	11.3 ± 2.5	3.2 ± 1.3	13.2 ± 2.5	5.3 ± 1.6	0.40	−0.13 ± 0.12
Ofloxacin (40)	10	42.4 ± 8.5	16.0 ± 3.3	48.6 ± 15.5	29.3 ± 7.5	0.60	−0.63 ± 0.12
Ceftriaxone (10)	8	66.3 ± 11.3	0.94 ± 0.69	59.3 ± 6.2	7.49 ± 7.60	0.12	−0.77 ± 0.17[g]

[a] NA, not applicable.
[b] $P < 0.05$ versus all treatment groups (all statistics were calculated by analysis of variance and Bonferroni correction).
[c] $P < 0.05$ versus rifampin at 5 mg/kg · h and rifampin at 20 mg/kg · h.
[d] ND, not done.
[e] Not significant versus rifampin at 5 mg/kg · h and ofloxacin at 10 mg/kg · h.
[f] Not significant versus rifampin at 10 mg/kg · h and ofloxacin at 40 mg/kg · h.
[g] $P < 0.05$ versus rifampin at 5, 10, and 20 mg/kg · h.

ously established to ensure rapid steady-state concentrations in serum and which was equivalent to the dose administered during 1 h (rifampin, ceftriaxone) or 2 h (ofloxacin) of continuous infusion, was administered as an intravenous bolus at the beginning of antibiotic therapy.

CSF and serum samples were collected at 1, 3, 5, and 7 h of therapy for the determination of bacterial titers and antibiotic concentrations in CSF. Pneumococcal titers in CSF were counted by plating 10 μl of undiluted CSF and serial 10-fold dilutions in saline on blood agar plates that were incubated overnight at 37°C at room air supplemented with 5% CO_2. The detection limit by this method was 10^2 CFU/ml. In order to account for the limited sensitivity of the test, we assigned the first sterile CSF sample obtained during therapy a value of 2 ($\log_{10} 10^2$), while subsequent sterile samples were assigned a value of 0 ($\log_{10} 1$). The bacterial titers at 0, 1, 3, 5, and 7 h were used to calculate the rate of bactericidal activity by linear regression analysis, which was expressed as $\Delta\log_{10}$ CFU per milliliter · hour.

Antibiotic concentrations in serum and CSF were determined by the agar-well diffusion technique in antibiotic medium 11 (Difco Laboratories), with *Staphylococcus epidermidis* ATCC 27626 used as the indicator strain for rifampin, *Bacillus subtilis* ATCC 6633 used as the indicator strain for ofloxacin, and *Escherichia coli* ATCC 10536 used as the indicator strain for ceftriaxone. For serum and CSF samples, different standard curves were constructed by using undiluted rabbit serum for the assay of serum and rabbit serum diluted 1:20 in saline for the assay of CSF (the latter approximating the protein concentration in CSF during meningitis). Lower levels of detectability in both assays were 0.25 μg/ml for rifampin and ceftriaxone and 1 μg/ml for ofloxacin.

Antibiotic concentrations and in vivo bactericidal activity. At all three doses of rifampin investigated, concentrations in serum indicated rapid achievement of steady state (Table 1), while the passage into CSF was slower (Table 1). The CSF-to-serum concentration ratio at 7 h, as an approximation of the steady-state condition, was about 0.2 for rifampin, which is comparable to that observed in humans (4, 14). This penetration rate was lower than that of ofloxacin (0.4 to 0.6; Table 1) but higher than that of ceftriaxone (0.12; Table 1). Steady-state rifampin concentrations in serum similar to those achieved in the rabbit model during the infusion of 5 mg/kg · h can be obtained in adults by administering a daily oral dose of approximately 900 mg. Peak concentrations of up to 20 μg/ml were obtained in human serum after the intravenous infusion of 600 mg, and were thus similar to the concentrations achieved in the rabbit model with the 10-mg/kg · h dose (12, 14). At a dose of 10 mg of rifampin per kg · h, the drug showed the most rapid rate of bactericidal activity in the CSF. Rifampin doses of 5 and 20 mg/kg · h were significantly ($P < 0.01$) less effective than the 10-mg/kg · h dose (Table 1). There was no positive correlation between the reduction of bacterial titers in CSF and mean concentrations in CSF over the dose range of rifampin examined ($r = -0.25$; not significant) (Fig. 1). This is in contrast to the results for the other classes of antibiotics examined in this model, including all β-lactams studied, for which a good correlation between concentrations and bactericidal activity in CSF can routinely be observed (20, 21).

Even at the most effective dose of rifampin (10 mg/kg · h), the drug was only approximately half as effective as ceftriaxone at the same dose in reducing bacterial titers in CSF (Table 1). This is in good agreement with the results of a recent study with the same model that used two penicillin- and cephalosporin-resistant pneumococci. In that study, rifampin showed moderate bactericidal activity compared with the bactericidal activity of vancomycin or a new quinolone (9). In an earlier study in the rabbit model of *Listeria monocytogenes* meningitis, rifampin was also significantly less effective than ampicillin or penicillin (18). Results of these studies suggest that rifampin is less rapidly bactericidal in CSF than comparison drugs. Results of the present study further suggest that killing rates comparable to those of β-lactam antibiotics cannot be obtained by increasing the dose of rifampin. On the contrary, CSF concentrations above those observed in humans with usual dosage regimens led to a decrease in bacterial killing (Table 1 and Fig. 1). A similar phenomenon has been described in vitro with streptococci and staphylococci after exposure to penicillins and has been termed the Eagle effect (7, 23). The underlying mechanism of this paradoxical effect of very high antibiotic concentrations under certain circumstances is still unknown. Reminiscent of the observations made in the present study with rifampin, very high concentrations of quinolones have been shown to reduce the killing of gram-negative bacteria.

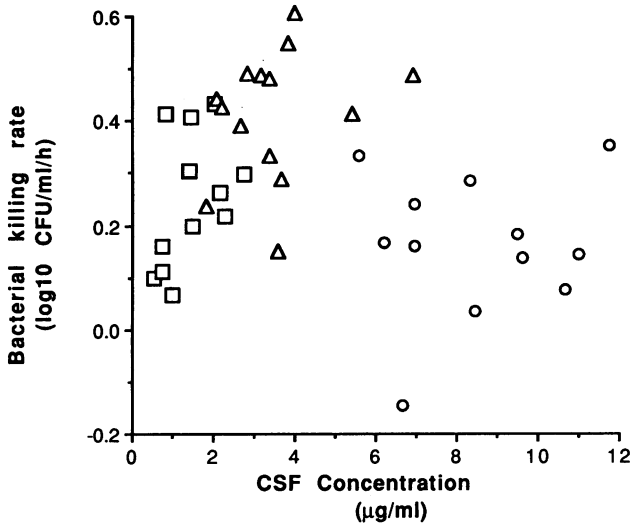

FIG. 1. Relationship between concentrations of rifampin in CSF and the bactericidal activity of the drug in CSF in experimental pneumococcal meningitis. Bactericidal activity was calculated from repeated quantitative bacterial cultures of CSF, while the concentrations of rifampin in CSF represent the arithmetic mean of sequential CSF concentrations determined during therapy, as described in the text. As a result of the decreasing bactericidal activity of rifampin at very high concentrations in CSF, there was no positive correlation between bactericidal activity in CSF and the concentrations of rifampin in CSF ($r = -0.25$; not significant). Rifampin was tested at 5 (□), 10 (△), and 20 (○) mg/kg · h.

This observation was explained by the assumption that ongoing protein synthesis of the bacteria is necessary for the bactericidal activity of quinolones (5, 19). It is unknown whether a similar mechanism may be responsible for the decreased killing of pneumococci with very high doses of rifampin.

The combination of rifampin and quinolones has been successfully used in the treatment of experimental and clinical endocarditis (6, 10). At doses of both drugs that lead to concentrations achievable in the sera and CSF of humans (5 mg of rifampin per kg · h plus 10 mg of ofloxacin per kg · h), the combination was not more active than rifampin alone at 5 mg/kg · h but, rather, tended to lead to reduced activity ($P = 0.056$ by Student's t test) (Table 1). The coadministration of rifampin at 10 mg/kg · h with ofloxacin at 40 mg/kg · h was more active than administration of rifampin alone, but was slightly less active than administration of ofloxacin alone at 40 mg/kg · h ($P = 0.06$ by Student's t test) (Table 1). Thus, the combination of rifampin plus ofloxacin at both concentrations examined did not show any synergism in the rabbit model of pneumococcal meningitis, but on the average, the killing rate of the combination was below the killing rate of the more active compound given alone. Similarly, rifampin decreased the bactericidal activities of quinolones against several bacterial species in vitro (16, 19). When rifampin was combined with either vancomycin or ceftriaxone for use in the treatment of experimental meningitis caused by β-lactam-resistant pneumococci, no synergistic or additive effect was observed for either combination, but vancomycin activity was reduced (9). Taken together, these data suggest that the addition of rifampin does not improve the bactericidal effects of other antibiotics in the treatment of pneumococcal meningitis.

In conclusion, the slow bactericidal activity observed in the present study qualifies rifampin as a less than optimal antibiotic for the therapy of meningitis caused by *S. pneumoniae*. Against pneumococci that are susceptible to β-lactams, rifampin appears to be considerably less active than ceftriaxone. The dose-response in vivo showed an unexplained paradoxical reduction in activity at very high concentrations, and the combination of rifampin and ofloxacin showed an indifferent effect, with a trend toward reducing the activity of the more active compound of the combination.

Part of this work was supported by a grant from Ortho-McNeil Pharmaceutical, Raritan, N.J. R. Nau was supported by a grant from the Walter-Marget-Foundation, Munich, Germany.

REFERENCES

1. **Bradley, J. S., and J. D. Connor.** 1991. Ceftriaxone failure in meningitis caused by Streptococcus pneumoniae with reduced susceptibility to beta-lactam antibiotics. Pediatr. Infect. Dis. J. **10**:871–873.
2. **Canton, E.** 1993. Cefotaxime breakpoint for *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **37**:616–617.
3. **Dacey, R. G., and M. A. Sande.** 1974. Effect of probenecid on cerebrospinal fluid concentrations of penicillin and cephalosporin derivatives. Antimicrob. Agents Chemother. **6**:437–441.
4. **D'Oliveira, J. J. G.** 1972. Cerebrospinal fluid concentrations of rifampicin in meningeal tuberculosis. Am. Rev. Respir. Dis. **106**:432–437.
5. **Dudley, M. N.** 1991. Pharmacodynamics and pharmacokinetics of antibiotics with special reference to the fluoroquinolones. Am. J. Med. **91**:S45–S50.
6. **Dworkin, R. J., B. L. Lee, M. A. Sande, and H. F. Chambers.** 1989. Treatment of right-sided Staphylococcus aureus endocarditis in intravenous drug users with ciprofloxacin and rifampicin. Lancet **ii**:1071–1073.
7. **Eagle, H., and A. D. Musselman.** 1949. The slow recovery of bacteria from the toxic effects of penicillin. J. Bacteriol. **58**:475–490.
8. **Figueiredo, A. M. S., J. D. Connor, A. Severin, M. V. Vaz Pat, and A. Tomasz.** 1992. A pneumococcal clinical isolate with high-level resistance to cefotaxime and ceftriaxone. Antimicrob. Agents Chemother. **36**:886–889.
9. **Friedland, I. R., M. Paris, S. Ehrett, S. Hickey, K. Olson, and G. H. McCracken, Jr.** 1993. Evaluation of antimicrobial regimens for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob. Agents Chemother. **37**:1630–1636.
10. **Kaatz, G. W., S. M. Seo, S. L. Barriere, L. M. Albrecht, and M. J. Rybak.** 1989. Ciprofloxacin and rifampin, alone and in combination, for therapy of experimental *Staphylococcus aureus* endocarditis. Antimicrob. Agents Chemother. **33**:1184–1187.
11. **Klugman, K. P.** 1990. Pneumococcal resistance to antibiotics. Clin. Microbiol. Rev. **3**:171–196.
12. **Kucers, A., and N. M. Bennett.** 1987. The use of antibiotics, 4th ed. William Heinemann Medical Books, London.
13. **Marton, A., M. Gulyas, R. Munoz, and A. Tomasz.** 1991. Extremely high incidence of antibiotic resistance in clinical isolates of Streptococcus pneumoniae in Hungary. J. Infect. Dis. **163**:542–548.
14. **Nau, R., H. W. Prange, S. Menck, H. Kolenda, K. Visser, and J. K. Seydel.** 1992. Passage of rifampicin into the cerebrospinal fluid of adults with uninflamed meninges. J. Antimicrob. Chemother. **29**:719–724.
15. **O'Keefe, P. T., and R. Bayston.** 1991. Pneumococcal meningitis in a child with a ventriculo-peritoneal shunt. J. Infect. **22**:77–79.
16. **Ratcliffe, N. T., and J. T. Smith.** 1984. Ciprofloxacin and ofloxacin exhibit a rifampicin-resistant bactericidal mechanism not detectable in other 4-quinolone antibacterial agents. J. Pharm. Pharmacol. **36**:59.
17. **Ring, J. C., K. L. Cates, K. K. Belani, T. L. Gaston, R. J. Sveum, and S. C. Marker.** 1979. Rifampicin for CSF shunt infections caused by coagulase-negative staphylococci. J. Pediatr. **95**:317–319.

18. **Scheld, W. M., D. D. Fletcher, F. N. Fink, and M. A. Sande.** 1979. Response to therapy in an experimental model of meningitis due to Listeria monocytogenes. J. Infect. Dis. **140:**287–294.
19. **Smith, J. T.** 1986. The mode of action of 4-quinolones and possible mechanisms of resistance. J. Antimicrob. Chemother. **18**(Suppl. D):21–29.
20. **Täuber, M. G., C. A. Doroshow, C. J. Hackbarth, M. G. Rusnak, T. A. Drake, and M. A. Sande.** 1984. Antibacterial activity of β-lactam antibiotics in experimental meningitis due to Streptococcus pneumoniae. J. Infect. Dis. **149:**568–574.
21. **Täuber, M. G., C. J. Hackbarth, K. G. Scott, M. G. Rusnak, and M. A. Sande.** 1985. New cephalosporins cefotaxime, cefpimizole, BMY 28142 and HR 810 in experimental pneumococcal meningitis in rabbits. Antimicrob. Agents Chemother. **27:**340–342.
22. **Viladrich, P. F., F. Gudiol, J. Linares, G. Rufi, J. Ariza, and R. Pallares.** 1988. Characteristics and antibiotic therapy of adult meningitis due to penicillin-resistant pneumococci. Am. J. Med. **84:**839–846.
23. **Yourassowsky, E., M. P. van der Linden, M. J. Lismont, F. Crockaert, and Y. Glupczynski.** 1988. Bactericidal effect and regrowth of Streptococcus faecalis exposed to amoxicillin following β-lactamase. Chemotherapy **34:**462–466.