Comparative Evaluation of Enoxacin, Ofloxacin, Ampicillin, and Chloramphenicol for Treatment of Experimental *Haemophilus influenzae* Pneumonia

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A murine model of bacteremic *Haemophilus influenzae* type b pneumonia was used to evaluate the therapeutic efficacies of the quinolone antimicrobial agents enoxacin and ofloxacin compared with those of ampicillin and chloramphenicol. Ampicillin-susceptible (AS) and ampicillin-resistant (AR) challenge strains were employed. Treatment with enoxacin or ofloxacin produced intrapulmonary killing of *H. influenzae* that was superior to that achieved with ampicillin ($P < 0.01$ to $P < 0.001$ for both AS and AR strains). Ofloxacin and enoxacin also provided killing greater than that with chloramphenicol for the AS strain ($P < 0.01$ to $P < 0.001$). For the AR strain, ofloxacin provided killing greater than that obtained with chloramphenicol ($P < 0.001$). Survival from AS strain pneumonia was 60% in enoxacin-treated and 78% in ofloxacin-treated animals compared with 41% for chloramphenicol-treated and 23% for ampicillin-treated groups. We conclude that enoxacin and ofloxacin may be effective antimicrobial agents in treating either AS or AR strains causing *H. influenzae* pneumonia.

Haemophilus influenzae is a frequent pulmonary pathogen and may cause life-threatening, invasive pneumonia (1, 5, 8–10, 14, 16). Results of recent studies suggest that the incidence of *H. influenzae* pneumonia among adults is increasing (5, 8, 16). With reported mortalities for this infection ranging from 33 to 57% (8, 16), studies to describe optimal therapy are well justified. Although ampicillin continues to be an important therapeutic agent for the treatment of serious *H. influenzae* respiratory infections, alternative therapeutic agents are needed for use in ampicillin-intolerant individuals and also for the treatment of β-lactamase-producing strains of *H. influenzae* (1, 9).

Enoxacin and ofloxacin are members of the quinolone class of antimicrobial agents which have demonstrated considerable in vitro activity against clinical *H. influenzae* isolates, including most ampicillin-resistant strains. For enoxacin, MICs for 90% of strains tested of 0.12 to 0.25 µg/ml have been reported for a series of *H. influenzae* isolates (2, 17), while for ofloxacin, MICs for 90% of strains tested of 0.06 to 1.56 µg/ml have been reported (13, 17). These in vitro observations suggest that enoxacin and ofloxacin should have considerable efficacy in the treatment of serious *H. influenzae* respiratory infections and provide initiative for experimental studies to address this issue. We have recently developed a murine model of experimental type b *H. influenzae* pneumonia in which such studies can be conducted (4). In this study we employed this experimental model to evaluate the therapeutic efficacy of enoxacin and ofloxacin in the treatment of *H. influenzae* pneumonia and to compare these efficacies with those of the conventional therapeutic agents ampicillin and chloramphenicol.

MATERIALS AND METHODS

Animals. Swiss Webster mice (both sexes; weight, 21 to 23 g) were obtained from Charles River Breeding Laboratories, Wilmington, Mass. Mice were housed in regulation animal quarters.

Bacteria. Two blood culture isolates of *H. influenzae* type b were provided by the Clinical Microbiology Laboratory, Children's Hospital, Boston, Mass. One strain was ampicillin susceptible (strain AS) and the other was ampicillin resistant (strain AR). The isolates were identified as *H. influenzae* by the demonstration of requirements for both X and V factors and by other standard criteria. Strain type was determined by slide agglutination with commercially prepared sera (Wellcome Research Laboratories, Beckenham, England [Div. Burroughs Wellcome Co.]). The absence of β-lactamase was confirmed with the phenol red test (3). Bacteria were preserved at −70°C on glass beads with brain heart infusion broth (GIBCO Diagnostics, Madison, Wis.) supplemented with 5% Filde enrichment (Difco Laboratories, Detroit, Mich.).

Measurements of MICs were done in brain heart infusion broth with 2% Filde enrichment by the tube dilution method (15). Each tube contained twofold dilutions of antibiotic and a final bacterial concentration of 10^6 CFU/ml. Tubes were incubated for 18 h at 37°C without CO_2 supplementation. The MIC was defined as the lowest concentration of antibiotic at which no growth was visible to the naked eye. For determination of MBCs, 0.01-ml fractions from tubes with no visible growth were plated onto chocolate agar and incubated overnight at 37°C in 5% CO_2–air. The MBC was defined as the lowest concentration of antibiotic resulting in killing of ≥99.9% of the original inoculum.

For the animal challenge studies, bacteria were inoculated into brain heart infusion broth with 5% Filde enrichment, incubated for 16 h at 37°C in a shaking water bath, washed twice, and suspended in 0.9% saline to the desired concentration, which was determined spectrophotometrically and confirmed by a serial dilution pour plate technique.

Antibiotics. Study drugs included ampicillin sodium (Wyeth Laboratories, Philadelphia, Pa.), chloramphenicol sodium succinate (Parke-Davis, Morris Plains, N.J.),

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TABLE 1. Microbiological and pharmacokinetic data for challenge strains and study drugs

Study drug (dose [mg/kg])	MIC/MBC (μg/ml) for:		Peak concn in serum (μg/ml)[a]	Half-life (min)
	Strain AS	Strain AR		
Ampicillin (100)	<0.25/<0.25	4/8	80[b]	13[b]
Chloramphenicol (50)	1/2	0.5/2.0	25[b]	38[b]
Enoxacin (20)	0.1/0.2	0.2/0.4	4.10 ± 0.45	68
Ofloxacin (20)	<0.03/<0.03	0.06/0.06	12.0 ± 2.5	52

[a] Peak concentrations in serum occurred 30 to 60 min after dosage. Mean ± standard error of the mean.

[b] Values were obtained from a previous study (4).

enoxacin (CI-919, AT-2266; Warner Lambert Co., Ann Arbor, Mich.), and ofloxacin (ORF 18489; Ortho Pharmaceutical Corp., Raritan, N.J.). Ampicillin and chloramphenicol were reconstituted according to instructions on the package insert and diluted in sterile water to a final concentration of 25 or 2.5 mg/ml, respectively. Enoxacin was supplied as a solution of 200 mg/ml and was diluted in water to a final concentration of 40 mg/ml. Powdered ofloxacin was dissolved in 0.1 N NaOH to a concentration of 10 mg/ml and thereafter titrated with concentrated HCl to neutral pH. Pharmacokinetic studies with enoxacin and ofloxacin were carried out with single subcutaneous (s.c.) injections of drug (20 mg/kg), after which groups of mice were sacrificed at 30, 60, 120, and 180 min. Blood was obtained by cardiac aspiration, and serum was separated and assayed for drug concentration. Agar diffusion (microbiologic) assays were carried out as described previously (7), and the half-life of enoxacin and ofloxacin in mice was calculated, also as described previously (7).

Experimental pneumonia. The method used for infecting murine lungs with *H. influenzae* has been described in detail elsewhere (4). Briefly, animals were anesthetized by intraperitoneal injection of Brevital (methohexital sodium; Eli Lilly & Co., Indianapolis, Ind.) and suspended vertically by hanging the lower incisor teeth on a wire hook and retaining the upper incisors with a taut rubber band. The oropharynx was transilluminated, and under direct visualization the trachea was cannulated with a blunt-tipped metal spinal needle (22 gauge), to which a microliter syringe containing the bacterial suspension was attached. After 40 μl of the suspension (approximately 10^9 CFU) was delivered into the trachea, the cannula-syringe apparatus was removed. The animals were maintained in a vertical position for 2 to 4 min and then placed at an angle of 45° until they were awake.

To evaluate relative efficacies of study drugs for intrapulmonary killing of bacteria, animals were treated with s.c. injections of enoxacin or ofloxacin (20 mg/kg) at 6 and 18 h after infection, or ampicillin (100 mg/kg) or chloramphenicol (50 mg/kg) at 6, 10, 14, and 18 h after infection. Control animals received four s.c. injections of 0.1 ml of isotonic saline. At 24 h after infection animals were killed with CO_2 and by cross-clamping of the trachea (for the prevention of agonal aspirations). The thorax was opened and heart blood was removed for culture. The thoracic contents were removed in toto, and the lungs were dissected free from the trachea and other structures and homogenized in 15 ml of sterile distilled water. Viable bacteria in the homogenate were quantitated by the serial dilution pour plate technique with brain heart infusion broth with 5% Filde enrichment. Results are expressed as log_{10} CFU of bacteria per mouse lung. Blood cultures were incubated for 18 h at 37°C and were then subcultured for confirmation of the presence or absence of *H. influenzae*.

For survival studies, antibiotic treatment was begun 6 h after infection and continued for 72 h. Regimens were as follows: enoxacin or ofloxacin, 20 mg/kg every 12 h; ampicillin, 100 mg/kg every 6 h; and chloramphenicol, 50 mg/kg every 6 h. Controls received injections of isotonic saline every 6 h. Cumulative survivals were recorded and compared at 24-h intervals.

Lung to serum ratio of enoxacin and ofloxacin. Lung penetration characteristics for the quinolone agents were determined at two separate time points after therapy. For the study, animals were given a single s.c. injection of enoxacin or ofloxacin and were then sacrificed at two different time intervals after injection. Serum was obtained by direct heart aspiration (as described above). Lungs were made oligemic by perfusion of the right ventricle with isotonic saline (4), removed, and homogenized in fivefold dilutions of isotonic saline (Polytron, Westbury, N.Y.). Enoxacin and ofloxacin concentrations in serum and lungs were determined by using reversed phase high-pressure liquid chromatography, and serum to lung concentration ratios were calculated by using lung concentrations adjusted for the dilution factor (×5).

Statistics. Data were analyzed by the two-tailed Student *t* test and the χ^2 test with the Yates's correction. *P* values of < 0.05 were considered significant.

RESULTS

In vitro and pharmacokinetic studies. Enoxacin and ofloxacin displayed high in vitro activities against both strains AR and AS of *H. influenzae* (Table 1). Enoxacin and ofloxacin dosages of 20 mg/kg resulted in peak concentrations in serum well above the MIC and MBC for the challenge strains. Based on these results the quinolones were employed in dosages of 20 mg/kg for the therapeutic trials. The dosages selected for ampicillin and chloramphenicol were those employed previously in this model (4).

Therapeutic efficacy in experimental pneumonia. All antibiotic regimens produced intrapulmonary killing of *H. influenzae* that was significantly greater than that observed in the control group (Table 2). Except for ampicillin treatment of strain AR, each regimen also reduced the incidence of bacteremia. The two quinolone agents were particularly effective in these studies. For strain AS, both antimicrobial agents demonstrated intrapulmonary killing that was significantly greater than that achieved with ampicillin or chloramphenicol. For strain AR, both quinolone agents provided killing superior to that with ampicillin, and for ofloxacin the killing was also superior to that with chloramphenicol.

To assess the therapeutic efficacies of study drugs over longer treatment periods and to provide confirmation of the lung clearance data, survival studies were carried out with strain AS. All treatment regimens prolonged survival from pneumonia compared with the control group (Table 3). As seen in the experiments described above, ampicillin was

TABLE 2. Efficacy of study drugs on clearance of *H. influenzae* from lungs and blood 24 h after experimental lung infection

Treatment (cumulative dosage [mg/kg per 24 h])	Viable bacteria in lungs ($\log_{10}$ CFU) in[a]:		Blood cultures (no. positive/total [%]) in:	
	Strain AS	Strain AR	Strain AS	Strain AR
Saline	7.59 ± 0.59^b	7.65 ± 0.57^b	15/18 (83)	13/16 (82)
Ampicillin (400)	5.17 ± 0.35^c	6.86 ± 0.60^c	10/16 (63)	12/14 (85)
Chloramphenicol (200)	$4.70 \pm 0.39^{d,e}$	4.86 ± 0.81^e	8/18 (44)	1/15 (7)
Enoxacin (40)	4.30 ± 0.55^e	4.64 ± 0.51^e	7/20 (35)	1/14 (7)
Ofloxacin (40)	3.39 ± 0.75	3.38 ± 0.53	1/17 (6)	0/14 (0)

[a] Values are expressed as mean ± standard deviation of $\log_{10}$ CFU of *H. influenzae* per mouse lung.
[b] Greater than treatment groups ($P < 0.01$ to 0.001).
[c] Greater than other treatments ($P < 0.01$ to 0.001).
[d] Greater than enoxacin ($P < 0.01$).
[e] Greater than ofloxacin ($P < 0.001$).

least effective, the quinolone agents were most effective, and chloramphenicol was intermediate in therapeutic efficacy (Table 3).

Lung to serum enoxacin and ofloxacin concentration ratios. We speculated that the high therapeutic efficacy of the quinolones may have resulted from efficient penetration into lung tissues. Accordingly, the drug concentration in lungs was determined and compared with the concomitant concentrations in serum. Because it was necessary to dilute and homogenize lungs for these assays, we utilized enoxacin and ofloxacin injections of 50 mg/kg. A total of 10 animals were studied at each time point for each drug. Mean (± standard error of the mean) concentrations (in micrograms per milliliter) in serum for enoxacin were 3.49 ± 0.57 (1 h) and 0.60 ± 0.10 (4 h). For lungs the corresponding concentrations were 9.06 ± 1.74 and 1.31 ± 0.31. Lung to serum ratios for enoxacin were thus 2.59 at 1 h and 2.18 at 4 h after the injections. For ofloxacin concentrations in serum were 7.57 ± 0.32 (1 h) and 0.71 ± 0.10 (4 h), while concentrations in lungs were 5.92 ± 0.91 (1 h) and 1.00 ± 0.35 (4 h). Lung to serum ratios for ofloxacin were 0.78 (1 h) and 1.40 (4 h).

DISCUSSION

Results of recent experimental studies suggest that quinolones are effective therapeutic agents for the treatment of certain respiratory infections. These studies used animal models of acute and chronic *Pseudomonas aeruginosa* pneumonia (6, 7), as well as *Legionella pneumophila* pneumonia (12). Although *H. influenzae* is a particularly common respiratory pathogen in humans (14), only limited clinical data are currently available to define the relative efficacy of quinolones compared with established antibiotics for the treatment of life-threatening *H. influenzae* pneumonia. Accordingly, we conducted a therapeutic trial of enoxacin and

TABLE 3. Survival from experimental pneumonia with strain AS of *H. influenzae*

Treatment (cumulative dosage [mg/kg per 24 h])	No. of infected animals	No. of survivors (% of total) at the following times (h) after infection:		
		24	48	72
Saline	22	10 (45)	5 (23)	1 (4)
Ampicillin (400)	22	14 (64)	8 (36)	5 (23)
Chloramphenicol (200)	22	18 (82)	13 (60)	$9 (41)^a$
Enoxacin (40)	25	25 (100)	20 (80)	$15 (60)^{b,c}$
Ofloxacin (40)	23	23 (100)	21 (91)	$18 (78)^{b,d,e}$

[a] Greater than control ($P < 0.01$).
[b] Greater than control ($P < 0.001$).
[c] Greater than ampicillin ($P < 0.02$).
[d] Greater than ampicillin ($P < 0.001$).
[e] Greater than chloramphenicol ($P < 0.02$).

ofloxacin compared with ampicillin and chloramphenicol in an experimental model of bacteremic *H. influenzae* type b pneumonia.

The therapeutic results obtained in this trial were encouraging and may have clinical relevance. For example, there is evidence that AR strains of *H. influenzae* are increasing in occurrence (1, 5, 8–10, 16). The therapeutic effectiveness of the quinolones against the AR challenge strain used in this study suggests that these agents may provide a new therapeutic option for treatment of AR strains. Also noteworthy in this study was the superior therapeutic efficacy of the quinolones compared with two other classes of antibiotics that are often employed for the treatment of *H. influenzae* infections. This observation was particularly evident when the quinolones were compared with ampicillin, even for the AS challenge strain.

To date limited published data are available regarding pulmonary distribution of quinolone agents. Recent published reports with animal models, however, suggest that these agents penetrate lung tissues well (11, 12). We speculate that the high therapeutic efficacy of quinolones in this study may be related to rapid and efficient penetration into lungs. The high lung to serum ratios observed for enoxacin and ofloxacin in this study (range, 0.77 to 2.74) are consistent with this possibility.

The eventual clinical role of enoxacin and ofloxacin in the treatment of serious *H. influenzae* respiratory infections remains to be defined. Furthermore, the unpredictable susceptibilities of non-*H. influenzae* respiratory pathogens (e.g., *Streptococcus pneumoniae*) to quinolones do not allow for extrapolation from the present data to generalizations on the treatment of pneumonia. Nevertheless, the encouraging observations made in this study do provide rationale for clinical trials of these quinolone agents in the treatment of advanced forms of *H. influenzae* respiratory infections, including acute pneumonia.

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