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Should *Pseudomonas aeruginosa* Isolates Resistant to One of the Fluorinated Quinolones Be Tested for the Others? Studies with an Experimental Model of Pneumonia

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A clinical isolate of *Pseudomonas aeruginosa* resistant to pefloxacin (Pef) but susceptible to ciprofloxacin (Cip) was studied to compare the in vitro and in vivo activities of Pef, ofloxacin (Ofl), and Cip. The time-kill curve method showed no bactericidal activity for Pef and Ofl, but a reduction of 4 $\log_{10}$ CFU/ml was achieved with Cip at 1 h. A model of experimental *P. aeruginosa* pneumonia was used to evaluate in vivo the relevance of the difference in susceptibility observed in vitro. At 36 h, a 100% cumulative survival rate was observed in Cip-treated rats, which was far higher than the survival rate obtained with Pef (53%) or Ofl (46%) ($P < 0.001$). At 4 h, no bacteremia was observed in Cip-treated rats, whereas 93% of the Pef-treated rats and 80% of the Ofl-treated rats were bacteremic ($P < 0.001$). The best pulmonary bacterial clearance was observed with Cip. Interestingly, Pef and Ofl, to which the strain was resistant in vitro, showed a fairly good in vivo activity despite sub-MIC concentrations. Cip was more effective than Pef and Ofl in terms of pulmonary and systemic bactericidal activity and provided the best survival rate in animals. We conclude that differences between the different quinolones in terms of the organism's sensitivity assessed in vitro may be relevant and that it might be useful to reconsider the use of a quinolone to which *P. aeruginosa* shows resistance if the organism shows sensitivity to no other agent.

Despite the use of broad-spectrum antimicrobial agents alone or in combination, nosocomial pneumonia, especially that due to *Pseudomonas aeruginosa*, is difficult to treat and has a very high mortality rate (50 to 80%), (4). Pefloxacin (Pef), ofloxacin (Ofl), and ciprofloxacin (Cip) are three fluorinated quinolones which are now widely used. It is of special interest that all of these antimicrobial agents are highly active against *P. aeruginosa*, including multiple-drug-resistant strains (2). Experimental models of *P. aeruginosa* pneumonia have previously demonstrated the efficacy of these fluorinated quinolones in normal as well as in neutropenic hosts (3, 6).

Susceptibility to one quinolone is usually considered representative of susceptibility to the others. However, some authors have reported the existence of *P. aeruginosa* strains resistant to Pef and Ofl but susceptible to Cip in vitro (5), although the relevance of these differences observed in vitro remains unclear and needs to be evaluated.

The objective of this study was to evaluate in vivo the significance of the differences in susceptibility to the different quinolones that were observed in vitro.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (body weight, 230 to 250 g) were obtained from Iffa Credo Laboratories (L'Arbresle, France). The animals were housed in standard wire-topped cages. Food and water were supplied ad libitum.

Bacteria and in vitro studies. A clinical isolate of *P. aeruginosa* resistant to Pef and Ofl but susceptible to Cip was used for this study. The MICs were determined in Mueller-Hinton broth supplemented with Mg^{2+} and Ca^{2+} by the macrotube dilution method with a standard inoculum of 5 $\log_{10}$ CFU/ml and are as follows: Pef, 8 mg/liter; Ofl, 8 mg/liter; and Cip, 0.5 mg/liter. The MICs were confirmed by the time-kill studies, which were performed with the three quinolones at concentrations that approximated the peak concentrations in serum. No bactericidal activity was observed with Pef and Ofl. However, a reduction of 4 $\log_{10}$ CFU/ml was observed with Cip during the first hour (Fig. 1).

Antibiotics. The three fluorinated quinolones used in this study, Cip, Ofl, and Pef, were obtained from Bayer Laboratories (Sens, France), Diamant Laboratories (Puteaux, France), and Rhône Poulenc Rorer Laboratories (Neuilly-sur-Seine, France), respectively.

Animal challenge studies. *P. aeruginosa* cells were incubated in 125 ml of tryptic soy broth at 37°C in a rotating shaking water bath. After 18 h, the culture was centrifuged (3,000 × g; 10 min), washed twice with phosphate-buffered saline (PBS [pH 7.4]), and resuspended in PBS.

Experimental pulmonary infection. Pneumonia was produced in rats according to the method described by Pennington and Ehrie (4). In brief, rats were placed under mild anesthesia with ether and a midline incision was made above the sternum; the trachea was then exposed by blunt dissection. A 28-gauge needle was inserted into the trachea, and 0.5 ml of the bacterial suspension was instilled (inoculum, 6×10^{10} CFU/ml). Preliminary data with this strain led us to use such a large inoculum in order to obtain a rapidly fatal experimental pneumonia.

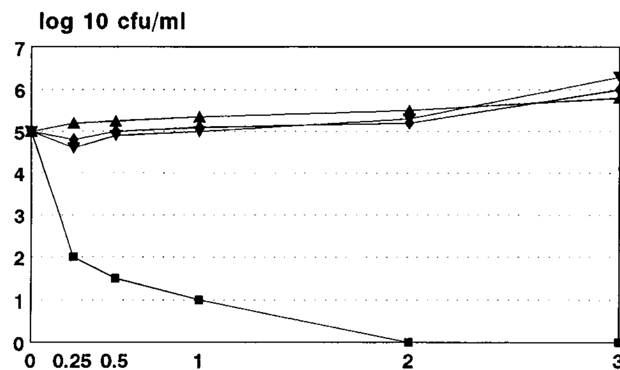

FIG. 1. Bactericidal activity of Cip, Ofl, and Pef against *P. aeruginosa* resistant to Pef but susceptible to Cip. ▲, control; ◆, Pef; ▼, Ofl; ■, Cip.

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TABLE 1. Pharmacokinetic parameters of Cip, Pef, and Ofl in noninfected male Sprague-Dawley rats after a single subcutaneous injection

Antibiotic (mg/kg of body wt)	Drug concn in[a]:					
	Serum (mg/liter) at min			Lung (mg/kg) at min		
	30	60	180	30	60	180
Pe (10)	4.3 ± 0.41	3.1 ± 0.22	1.31 ± 0.19	7.3 ± 0.62	5.2 ± 0.51	2.32 ± 0.13
Ofl (10)	3.27 ± 0.16	2.39 ± 0.47	0.98 ± 0.22	4.03 ± 0.63	3.06 ± 0.46	1.19 ± 0.14
Cip (20)	4.63 ± 0.39	3.13 ± 0.30	1.47 ± 0.22	8.03 ± 0.57	5.85 ± 0.56	2.56 ± 0.16

[a] Values are means ± standard errors of five individual determinations.

Therapy. Therapy was initiated 2 h after intratracheal challenge when the pneumonia was well established. Antibiotics were administered to groups of rats (minimum group of 15) subcutaneously. Rats received a single injection of Pef (10 mg/kg of body weight), Ofl (10 mg/kg), Cip (20 mg/kg), or saline. Antibiotics were resuspended in saline in order to obtain a concentration of 2 mg/ml.

Measurement of antibiotic concentrations. Noninfected rats were sacrificed 30, 60, and 180 min after drug administration. Blood specimens were collected by cardiac puncture. Lungs were aseptically removed from exsanguinated rats, weighed, and homogenized in 1 ml of PBS. Antibiotic concentrations were determined by high-performance liquid chromatography.

Survival study. Animals were observed every 6 h for a period of 36 h after the bacterial challenge.

Pulmonary and systemic clearance study. Animals were killed immediately (zero hour) and 4 h after treatment. Rats were exsanguinated by cardiac puncture. The lungs were aseptically removed and then homogenized in Tris-buffered saline, and viable bacteria were numerated by plating 0.1 ml of serial 10-fold dilutions of the homogenates on agar plates. Results are expressed as CFU per milliliter of lung homogenate. Blood samples were taken for culturing, and viable bacteria were numerated on agar plates.

Statistics. Differences in survival rates were compared by the chi-square analysis with Yates' correction. The paired Student's t test was employed for analysis of quantitative bacteriology.

RESULTS

Pharmacokinetics study. The concentrations of drugs in serum samples and lung homogenates 30, 60, and 180 min after injection are depicted in Table 1.

Therapeutic efficacy in experimental pneumonia. (i) Survival study. At 36 h after initiation of therapy, no death (0 of 18 rats) occurred in Cip-treated animals. This is significantly different from the survival observed in the Pef-treated group (8 of 15 rats; $P < 0.001$), Ofl-treated group (7 of 15 rats; $P < 0.001$), and saline-treated group (1 of 15 rats; $P < 0.001$) (Table 2).

(ii) Systemic bactericidal activity. At 4 h after initiation of therapy, no positive blood culture was observed from the Cip-treated group (0 of 18 rats). This is significantly different from positive blood cultures observed from the Pef-treated group (14 of 15 rats; $P < 0.001$), Ofl-treated group (12 of 15 rats; $P < 0.001$), and saline-treated group (15 of 15 rats; $P < 0.001$) (Table 3).

(iii) Pulmonary bactericidal activity. At 4 h after initiation of therapy, 6.4 ± 0.43 $\log_{10}$ CFU of viable bacteria per ml were recovered from Cip-treated group lung homogenate, which is significantly different from the findings for the Pef-treated group (7.7 ± 0.48 $\log_{10}$ CFU/ml; $P < 0.01$), Ofl-treated group (7.6 ± 0.47 $\log_{10}$ CFU/ml; $P < 0.01$), and saline-treated group (8.8 ± 0.56 CFU/ml; $P < 0.001$) (Table 4).

DISCUSSION

The objective of this study was not to compare the activities of different quinolones but to investigate in vivo the relevance of differences in susceptibility observed in vitro. Indeed, the goal was to determine if, for *P. aeruginosa*, susceptibility to one quinolone is reliably predictive of susceptibility to the others. For this purpose, a clinical isolate of *P. aeruginosa* resistant to Pef but susceptible to Cip was used in an experimental model of pneumonia in rats.

Cip exhibited the best survival rate (100% at 36 h) and the best systemic bactericidal activity (no bacteremia at 4 h) compared with Pef and Ofl. The activity of Cip assessed by pulmonary bacterial clearance was less impressive, but this could be explained by the size of the inoculum used in this experiment. Finally, these results are consistent with the in vitro data.

A similar finding has recently be reported with fluoroquinolones. It has been demonstrated that in vitro susceptibility tests were not reliably predictive of the efficacy of fluoroquinolones for treating experimental pneumococcal pneumonia in mice; temafloxacin was found to be much more effective than Cip and Ofl in both acute and subacute *Streptococcus pneumoniae* models despite similar in vitro activities (1). This can be explained by differences in pharmacokinetic profiles in serum and lung samples between noninfected and infected animals and differences in maximum serum drug concentrations in serum and lung samples. Levels of tissue penetration in both noninfected and infected mice were higher with temafloxacin than with Cip and Ofl (7).

In our opinion, our results could not be explained by differences in pharmacokinetics. Indeed, the serum and lung homogenate concentrations obtained at the different times of investigation were quite similar for Cip and Pef.

Another point needs to be discussed: Pef and Ofl, for which the strain was resistant in vitro, exhibited a weak systemic bactericidal activity, with 93.3% and 80% positive blood cultures, respectively (Table 3). In contrast, the initially deposited viable bacteria within the lungs were reduced by 1 $\log_{10}$ CFU/ml at 4 h in Pef- and Ofl-treated animals (Table 4).

TABLE 2. Survival study

Treatment group	No. of rats surviving/ no. tested (%) at 36 h
Saline	1/15 (6.6)
Cip[a]	18/18 (100)
Ofl	7/15 (46.66)
Pef	8/15 (53.33)

[a] $P < 0.001$ versus (i) Ofl and (ii) Pef.

TABLE 3. Systemic bactericidal activity: positive blood cultures at 4 h

Treatment group	No. of positive blood cultures/no. tested (%)
Saline	15/15 (100)
Cip[a]	0/18 (0)
Ofl	12/15 (80)
Pef	14/15 (93.3)

[a] $P < 0.001$ versus (i) Ofl and (ii) Pef.

TABLE 4. Pulmonary bactericidal activity: viable bacteria in lung homogenate at 4 h

Treatment group (no. of rats)	No. of viable bacteria ($\log_{10}$ CFU/ml)
Control (15)	8.8 ± 0.56
Saline[a] (15)	8.5 ± 0.84
Cip[b] (18)	6.4 ± 0.43
Ofl[c] (15)	7.6 ± 0.47
Pef (15)	7.7 ± 0.48

[a] $P < 0.02$ versus (i) Ofl and (ii) Pef.
[b] $P < 0.001$ versus (i) saline, (ii) Ofl, and (iii) Pef.
[c] Ofl versus Pef, not significant.

Moreover, almost 50% survival was observed with these drugs (Table 2) despite sub-MIC concentrations (Table 1). This fairly good in vivo activity probably reflects a sub-MIC effect. These results mean it might be useful to try a quinolone to which for *P. aeruginosa* shows resistance if the organism shows sensitivity to no other agent.

These in vivo results seem to confirm the in vitro data and demonstrate that differences in the in vitro sensitivity of *P. aeruginosa* to the different quinolones may exist, which may have therapeutic implications. However, this conclusion should be confirmed with other experimental models.

In conclusion, it may be worthwhile to test *P. aeruginosa*'s sensitivity to the different quinolones available when resistance to one of these agents is observed in vitro. In cases in which *P. aeruginosa* shows resistance to the different agents available, these results may lead to reconsideration of the use of a quinolone to which *P. aeruginosa* shows resistance.

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