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Resistance Occurring after Fluoroquinolone Therapy of Experimental *Pseudomonas aeruginosa* Peritonitis

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Resistance emerging after fluoroquinolone therapy was investigated in a murine model of *Pseudomonas aeruginosa* infection. Mice were infected intraperitoneally by one of six strains and treated with pefloxacin or ciprofloxacin. In mice challenged with a low inoculum (1.6×10^5 CFU), no resistance occurred. With a higher inoculum (1.5×10^8 CFU) and after a single dose of antibiotic, posttherapy (PT_1) strains with decreased susceptibility to quinolones (4- to 32-fold less) were isolated at a variable rate. The presence of talcum (125 mg) in the peritoneal cavity increased the risk of resistance after therapy. Pefloxacin (25 or 200 mg/kg) and ciprofloxacin (25 mg/kg) yielded similar resistance rates (61 to 77%), but ciprofloxacin (10 mg/kg) produced more resistance (83%) than did ciprofloxacin (50 mg/kg) (44%) ($P < 0.02$). Combined with a quinolone, ceftazidime ($P < 0.001$) or amikacin ($P < 0.01$), but not piperacillin, reduced the emergence of resistance. After several doses of ciprofloxacin, it was found that 25-mg/kg doses every 12 h produced more resistance than did 25-mg/kg doses every 8 h or 50-mg/kg doses every 12 h. Compared with the preceding experiments using parent strains, ciprofloxacin and pefloxacin were less efficient in killing bacteria in mice infected with PT_1 strains. Moreover, in one of these mice, a highly resistant PT_2 strain (64-fold MIC increase for the quinolones) emerged. Besides increased MICs of the quinolones, there was a two- to eightfold increase in imipenem MIC for all PT_1 and PT_2 strains without alteration of other β-lactam and aminoglycoside susceptibility. Some PT_1 strains also showed a decreased susceptibility to trimethoprim and chloramphenicol. During therapy with a quinolone, resistance can emerge rapidly, especially when there is a large number of bacteria or a foreign body present. This risk may depend on the dosing schedule and may be reduced by combined therapy.

During the past decade, the emergence of resistance after therapy with newer β-lactam compounds has been an increasing concern (12). In our laboratories, we have reproduced this phenomenon by using an experimental model of *Enterobacter cloacae* infection (6a). We showed that the emergence of resistance varied with the β-lactam compound given to the animal. When the newer fluoroquinolones appeared, it was hoped that the development of resistance during therapy would be avoided (6). As a result of the improved antibacterial activity of drugs such as pefloxacin and ciprofloxacin, the resistant variants found in the bacterial populations, although less susceptible than the parent strains, might still be regarded as susceptible to these drugs (6). Unfortunately, this did not hold true, and several preliminary therapeutic trials have shown that resistant strains emerging after therapy could generate therapeutic difficulties, especially in *Pseudomonas* infections (11, 16). In this paper, we compared the abilities of pefloxacin and ciprofloxacin to produce resistance in our murine model, with six strains of *Pseudomonas aeruginosa* under different conditions.

MATERIALS AND METHODS

Antimicrobial agents. Pefloxacin was provided by Rhône-Poulenc, Paris, France, and ciprofloxacin was provided by Bayer AG, Leverkuusen, Federal Republic of Germany. The other antimicrobial agents were obtained from their respective manufacturers. Working antibiotic solutions were freshly prepared from powders of known potency according to the recommendations of the manufacturers.

Bacterial strains. Strains 1 to 6 were clinical isolates of *P. aeruginosa* obtained from infected patients, admitted to the University Hospital of Geneva but not treated with a quinolone. Strains were maintained in skim milk and stored at −70°C. At the start of the study, the strains were thawed, plated on human blood agar to check purity, and grown overnight in L broth (Bacto Tryptone [Difco Laboratories, Detroit, Mich.], 10 g; NaCl, 10 g; yeast extract, 5 g; distilled water, 1,000 ml).

Susceptibility testing. Antimicrobial activity was determined by two methods. The first was a microdilution technique (15) in Mueller-Hinton broth. The second used antibiotic-containing gradient plates (2) and was performed as follows. Strains were grown overnight in L broth, and 100 μl of bacterial culture (corresponding to 2×10^8 to 3×10^8 CFU) was uniformly spread over L agar containing an antibiotic gradient. Gradients were prepared in square petri dishes (9 by 9 cm) and provided antibiotic concentrations ranging linearly from 0 to the chosen maximum. After incubation for 24 h at 35°C, bacterial growth was examined. Typical growth aspects on the gradients include confluent growth at lower antibiotic concentrations and single colonies at higher concentrations. The sharp limit between these two zones is referred to as the boundary (2). Antibiotic-containing gradient plates allowed the definition of two levels of antibiotic activity, one at the boundary concentration and the other corresponding to the minimal concentration inhibiting all visible growth (no-growth concentration).

Frequency of resistant variants. One hundred microliters of an overnight L broth culture, i.e., 2×10^8 to 8×10^8 CFU,

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TABLE 1. Susceptibility testing by microdilution technique of six strains of *P. aeruginosa* before therapeutic exposure

Antibiotic	MIC (μg/ml) for strain:					
	1	2	3	4	5	6
Ciprofloxacin	0.1	0.2	0.05	0.1	0.1	0.1
Pefloxacin	2	4	2	2	1	1
Norfloxacin	0.5	1	0.25	0.25	0.5	0.25
Ofloxacin	1	2	1	2	1	1
Nalidixic acid	64	512	128	128	128	128
Aztreonam	16	32	8	16	16	16
Cefotaxime	16	32	16	16	16	16
Ceftriaxone	16	64	8	16	16	16
Ceftazidime	2	4	2	2	2	2
Imipenem	2	1	4	1	2	1
Piperacillin	8	8	4	8	8	8
Amikacin	8	8	4	8	8	4
Gentamicin	8	4	4	8	8	4
Chloramphenicol	64	128	64	64	64	64
Trimethoprim	128	256	128	128	128	128

was uniformly plated on agar containing pefloxacin or ciprofloxacin at a concentration four or eight times higher than the initial corresponding MIC. After 48 h of incubation, colonies were counted and compared to the inoculum.

Animal model. Swiss ICR female mice, weighing 20 to 30 g, were conditioned for 1 week after receipt from the breeders and kept in conventional cages with free access to water and antibiotic-free chow. Inoculum was prepared from an overnight culture in L broth after proper dilution in 0.9% NaCl solution. Peritonitis was established by intraperitoneal injection of 1 ml of a bacterial suspension with or without 125 mg of talcum. Talcum, or magnesium hydropolysilicate [$Mg_3Si_4O_{10}(OH)_2$], was used as a foreign body in the peritoneal cavity. Such a suspension, 250 mg of talcum per ml in 0.9% NaCl solution, provides a 0.16 mM concentration of free Mg^{2+} ions, as measured in an atomic absorption spectrophotometer (model AA-1475; Varian International S.A, Geneva, Switzerland). This concentration does not significantly influence the antibacterial activity of pefloxacin and ciprofloxacin (1).

At 2 h after bacterial challenge, a dose of antibiotic was administered subcutaneously. In the single-dose treatments, mice were sacrificed by hyperanesthesia 22 h after the dose. In the multiple-dose treatments, equal doses of antibiotic were given every 8 or 12 h. When the animals survived, the treatment was discontinued after injection 5, and the mice were sacrificed 24 h later. In all animals, the peritoneal fluid was sampled shortly after death through an iliac incision (2 by 3 mm) and plated on antibiotic-free Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) for colony counting after appropriate dilutions, whereas the rest of the sample was placed into antibiotic-free L broth and allowed to grow overnight for further susceptibility testing on antibiotic-containing gradient plates. After comparing data from treated animals and untreated control animals, we defined a significant shift toward resistance as an increase of the boundary concentration of at least fourfold. Those strains showing a significant shift after a single therapeutic exposure were referred to as PT_1 (posttherapy) strains. Some of the PT_1 strains were reinoculated into naive mice and exposed to a second course of antibiotic therapy. If there was a further increase of the boundary concentration by at least fourfold, the strain was referred to as a PT_2 strain.

Pharmacokinetics. Antibiotic concentrations in the peritoneal fluid and total blood were determined by a microbiological assay, a disk diffusion method with *Escherichia coli* or *Bacillus subtilis* test organisms, as described previously (7). The apparent half-life of antibiotic activity in the peritoneal fluid was estimated from three or four time points after administration of pefloxacin or ciprofloxacin.

Statistical analysis. Chi-square analysis was used for comparing the resistance rates, and Student's *t* test was used for comparing the bacterial counts.

RESULTS

Susceptibility testing before therapy. The initial antibiotic susceptibility of the six strains is shown in Table 1. MICs ranged from 1 to 4 μg/ml for pefloxacin and from 0.05 to 0.2 μg/ml for ciprofloxacin. As expected, the MICs of nalidixic acid were higher by several orders of magnitude. Chloramphenicol and trimethoprim were found to be poorly active (MIC, >64 μg/ml).

Susceptibility testing with the antibiotic-containing gradient plates is presented in Table 2. Within 1 dilution, the boundary concentration corresponded to the MIC obtained by the microdilution technique. Beyond the boundary, single colonies were observed with all six strains (except strain 6 on the ciprofloxacin gradient). The bacterial populations contained resistant variants at similar frequencies, within 1 order of magnitude for both antibiotics. The no-growth concentration determined on the pefloxacin gradients was two to three times higher than the corresponding boundary concentration for strains 3 and 6 and six to eight times higher than the boundary concentrations for the other strains. The ciprofloxacin no-growth concentration was 4 to 12 times higher than the boundary concentration.

Control animals infected with the parent strains. Seventy-five infected, but untreated, mice were used as controls. An inoculum of 1.6×10^5 CFU (range, 0.7×10^5 to 2.4×10^5) supplemented with 125 mg of talcum produced a lethal peritonitis within 24 h in all animals. At the time of death, the volume of peritoneal fluid was estimated at about 0.5 ml, in which 1×10^7 to 3.2×10^8 CFU/ml were enumerated. An inoculum of 1.5×10^8 CFU (range, 0.85×10^8 to 3.3×10^8), with or without talcum, produced a still more severe peritonitis, with death occurring in less than 16 h. The peritoneal cavity contained approximately 1 ml of purulent fluid in which bacterial counts ranged from 1.05×10^{10} to 5.5×10^{11} CFU/ml. For a given inoculum, the six strains yielded similar peritoneal bacterial counts at autopsy. Since the number of peritoneal bacteria at the time of death was always higher by several logs than the inoculum, an actual infection occurred in all cases.

Animals with single-dose therapy. A total of 225 mice received a single dose of antibiotic in either a monotherapy or a combined therapy (Table 3). Each of the six strains was exposed to each therapeutic regimen in three mice. The therapeutic activity was assessed by the number of CFU per milliliter in the peritoneal fluid. Compared with the control animals, a mean log decrease of 2.46 to 7.30 was observed in the treated animals. No resistance was found after a single dose of pefloxacin (25 mg/kg), in mice challenged with 1.6×10^5 CFU plus talcum. When the inoculum was 1.5×10^8 CFU, resistance emerged, especially when the bacterial inoculum was supplemented with talcum (Table 3). A similar number of mice with PT_1 strains appeared with pefloxacin (25 or 200 mg/kg), even though the latter dosage was more efficient in bacterial killing (Table 3, column 4).

Ciprofloxacin (25 mg/kg) yielded a resistance rate similar to that of pefloxacin at the same dosage (a nonsignificant

TABLE 2. Susceptibility testing with antibiotic-containing gradient plates and frequency of resistant variants of six strains of *P. aeruginosa* before therapeutic exposure

Strain	Antibiotic	Boundary concn[a] (μg/ml)	No-growth concn[b] (μg/ml)	Frequency of resistant variants at:	
				4× MIC	8× MIC
1	Pefloxacin	2.5	15	7.5×10^{-8}	$<10^{-8}$
	Ciprofloxacin	0.1	0.5	2.9×10^{-7}	3.2×10^{-8}
2	Pefloxacin	6	45	1.5×10^{-8}	$<10^{-8}$
	Ciprofloxacin	0.2	0.8	5.6×10^{-8}	$<10^{-8}$
3	Pefloxacin	2	4	8.3×10^{-7}	$<10^{-8}$
	Ciprofloxacin	0.05	0.6	8.7×10^{-7}	5.4×10^{-8}
4	Pefloxacin	3	25	1.6×10^{-7}	$<10^{-8}$
	Ciprofloxacin	0.1	0.8	3.2×10^{-7}	7.8×10^{-8}
5	Pefloxacin	3	25	2.0×10^{-7}	5.1×10^{-8}
	Ciprofloxacin	0.1	0.7	1.4×10^{-7}	5.7×10^{-8}
6	Pefloxacin	2	6	7.3×10^{-8}	$<10^{-8}$
	Ciprofloxacin	0.1	0.1	3.3×10^{-8}	$<10^{-8}$

[a] Boundary concentration, Antibiotic concentration corresponding to the boundary in the gradient plates.
[b] No-growth concentration, Minimal antibiotic concentration inhibiting all visible growth on the gradient plates.

difference), but the former produced more bacterial killing than the latter ($P < 0.01$). In contrast with the pefloxacin findings, the resistance rate after ciprofloxacin therapy was dose dependent; 50 mg/kg produced less resistance than did 25 or 10 mg/kg. Ciprofloxacin (50 mg/kg) selected less resistance ($P < 0.01$) and produced more bacterial killing ($P < 0.01$) than did pefloxacin (200 mg/kg).

Combinations including amikacin or ceftazidime greatly limited the emergence of resistance, especially the latter antibiotic. In contrast, combinations including piperacillin did not reduce the resistance rates of the corresponding monotherapies (nonsignificant differences). Amikacin, ceftazidime, and piperacillin given alone produced no resistance (data not shown).

Table 3 also shows that the ability to produce resistance was not similar for the six strains. Resistance emerged only once with strain 6, whereas the highest resistance rate was recorded with strains 4 and 5.

Animals with multiple-dose therapy. A total of 45 mice, challenged with 0.85×10^8 to 3.3×10^8 CFU of the parent strains, were treated with 25 or 50 mg of ciprofloxacin per kg every 8 or 12 h (Table 4). A total of 33 of these mice died between dose 3 and 4, whereas the remaining 12 mice were sacrificed after dose 5. In three mice, which were given five 50-mg/kg doses, the peritoneal fluid yielded no growth. The resistance rate depended on the dosing interval. So ciprofloxacin, given 25 mg/kg every 12 h, produced resistance in 50% of the animals, whereas the same regimen with 8-h intervals yielded no resistance ($P < 0.01$). In addition, with 12-h intervals, the resistance rate was lower after 50 mg/kg than after 25 mg/kg ($P < 0.01$).

In another set of experiments, 24 mice were challenged with PT_1 isolates obtained from strains 1, 2, and 4 (inoculum, 0.6×10^8 to 1.8×10^8 CFU) plus talcum (125 mg). In the six untreated control mice, the experimental infection was comparable with that obtained with the parent strains in the time

TABLE 3. Analysis of bacterial population in the peritoneal fluid of mice after single-dose therapy[a]

Antibiotic	Dosage (mg/kg)	Talcum (125 mg)	Mean $\log_{10}$ CFU decrease[b]	No. of mice with PT_1 strains						*P* values (chi square)
				Parent strain						
				1	2	3	4	5	6	
Monotherapy										
Pefloxacin	25	−	2.46	2	0	0	2	3	0	<0.05
	25	+	3.11	3	3	1	3	3	0	NS[c]
	200	+	6.36	1	3	3	3	3	1	
Ciprofloxacin	10	+	5.30	3	3	3	3	3	0	<0.02
	25	+	6.30	3	2	2	2	2	0	
	50	+	7.30	1	2	2	2	1	0	
Combined therapy										
Pefloxacin	25									
Ceftazidime	50	+	5.55	0	0	0	0	0	0	<0.01
Amikacin	15	+	3.88	2	3	0	1	1	0	NS
Piperacillin	50	+	3.11	3	0	3	3	3	0	
Ciprofloxacin	25									
Ceftazidime	50	+	6.1	0	0	0	0	0	0	<0.05
Amikacin	15	+	5.1	0	0	0	1	0	0	
Piperacillin	50	+	7.1	1	3	0	0	1	0	

[a] Mice inoculated with 0.85×10^8 to 3.3×10^8 CFU of the parent strain. Eighteen mice were used for each therapeutic regimen.
[b] Compared with values from control animals.
[c] NS, Not statistically significant.

TABLE 4. Therapeutic effects of multiple doses of ciprofloxacin in mice[a]

Ciprofloxacin dose (mg/kg)	Dosing interval (h)	Total no. of mice treated	No. of mice surviving after dose 5	No. of mice with PT_1 strains from parent strain:						Mean $\log_{10}$ CFU decrease in peritoneal fluid[b]
				1	2	3	4	5	6	
25	12	18	1	2	3	0	2	1	1	6.2
25	8	9	3	0	0	ND[c]	0	ND	ND	7.8
50	12	18	8	0	1	0	0	0	0	7.2

[a] Mice inoculated with 0.85×10^8 to 3.3×10^8 CFU of the indicated parent strain and 125 mg of talcum.
[b] Compared with values from control animals.
[c] ND, Not determined.

to death and macroscopic aspect of the peritoneal cavity at autopsy. However, bacterial counts in the peritoneal fluid at the time of death were lower (5×10^8 to 1.2×10^{10} CFU/ml) than those found after inoculation with the parent strains ($P < 0.01$). The other 18 mice were treated with several doses of pefloxacin (50 mg/kg) or ciprofloxacin (25 mg/kg), with dosing intervals of 8 h (Table 5). All mice but one died between dose 2 and 5. Compared with the corresponding control animals, the decrease of bacterial counts in the peritoneal fluid produced by ciprofloxacin, given 25 mg/kg every 8 h, was smaller by at least 3 logs in mice challenged with PT_1 strains than in mice challenged with parent strains ($P < 0.001$). A PT_2 strain was isolated only once, in a ciprofloxacin-treated mouse.

Antibiotic susceptibility of PT_1 and PT_2 strains. MICs were determined by microdilution method and compared with the corresponding parental MICs (Table 6). PT_1 strains were characterized by 4- to 32-fold increases of the quinolone MICs, which paralleled 2- to 8-fold increases of imipenem MICs. In addition, PT_1 strains obtained after combinations including piperacillin (Table 6, columns 3 and 4) exhibited 4- to 16-fold increases of chloramphenicol and trimethoprim MICs. The PT_2 strain was characterized by a high level of resistance to the five quinolones tested, associated with a decreased susceptibility to imipenem. Most of the PT_1 and the PT_2 strains were found to be two to four times more susceptible to amikacin and gentamicin than the parental strain was.

Antibiotic assays. Antibiotic concentrations in blood and peritoneal fluid, and apparent half-lives of antibiotic activity are presented in Table 7. This table does not include results previously reported with amikacin (15 mg/kg) (7). Ceftazidime (50 mg/kg) produced higher antibiotic concentrations in blood and peritoneal fluid than did the same dosage of piperacillin, in which the antibiotic concentrations were occasionally lower than the MICs.

DISCUSSION

As has been found in vitro (14), this model of pseudomonal peritonitis allowed the rapid emergence of resistant organisms, provided that the inoculum was high enough. The presence of talcum in the peritoneal cavity significantly increased the risk of resistance after therapy. Since ionization of talcum was minimal under our experimental conditions, this material probably acted as a foreign body. When infected foreign bodies have a profound effect on the activity of antimicrobial agents, it is often necessary to remove the foreign material to cure the infection. The reasons for foreign bodies potentiating infection and generating therapeutic difficulties remain unclear, but localized impairments of host defenses have been implicated (17). The present study supports the concept that the emergence of resistance might represent another contributive factor in the therapeutic failures of infected foreign bodies.

The resistance probably results from the rapid selection of the resistant variants which were seen on the antibiotic-containing gradient plates in almost all cases. The frequency of variants at four and eight times the MIC (Table 2) accounted for the fact that no resistance would be expected in animals inoculated with only 10^5 CFU. However, the resistance rate did not correlate accurately with the frequency of the variants; for instance, strain 6 exhibited similar resistant variants but less emergence of resistance than did strain 2.

Actually, the occurrence of resistance followed the susceptibility of the resistant variants more closely than their frequency. Thus, resistance occurred rarely with strains 3 and 6, in which the no-growth concentrations of pefloxacin were 4 and 6 μg/ml, respectively (Table 2). On the other hand, resistance emerged regularly in animals inoculated with strains 2, 4, and 5, which included the most resistant variants. However, when the levels of resistance of the preexisting variants were considered together with the antibiotic concentrations measured in the peritoneal fluid, surprising findings appeared. At a dosing of 25 mg/kg, pefloxacin and ciprofloxacin produced similar resistance rates (respectively, 13 of 18 and 11 of 18 after an inoculum of 1.5×10^8 CFU with talcum) and similar pharmacokinetic profiles (Table 7). In contrast, the no-growth concentration of pefloxacin was 7 to 60 times less active in vitro than that of ciprofloxacin (Table 2). However, in humans, pefloxacin is converted into norfloxacin (8). The same holds true in mice, but the conversion is less complete (8). Table 1 shows norfloxacin to be two- to eightfold more active than pefloxacin was against *P. aeruginosa*. Hence the difference in activity of pefloxacin and ciprofloxacin in vitro may not accurately reflect in vivo activity. Such a discrepancy was

TABLE 5. Therapeutic effects of multiple-dose therapy in 18 mice[a]

Antibiotic	Dose (mg/kg)	Dosing interval (h)	No. of mice			Mean $\log_{10}$ CFU decrease in peritoneal fluid[b]
			Total treated	Surviving after dose 5	With PT_2 strain	
Ciprofloxacin	25	8	9	1	1	3.87
Pefloxacin	50	8	9	0	0	1.54

[a] Mice inoculated with 0.6×10^8 to 1.8×10^8 CFU of PT_1 strain and 125 mg of talcum.
[b] Compared with values from control animals.

TABLE 6. Susceptibility testing of PT_1 and PT_2 strains obtained after different treatments

Antibiotic	Increase (fold) in MICs[a]				
	PT_1/Pefloxacin (12)[b]	PT_1/Ciprofloxacin (10)	PT_1/Pefloxacin + piperacillin (5)	PT_1/Ciprofloxacin + piperacillin (3)	PT_2/Ciprofloxacin (1)
Ciprofloxacin	4–32	8–32	8–16	8–16	64
Pefloxacin	4–16	8–16	2–16	4–16	64
Norfloxacin	4–16	8–16	8–16	8–16	64
Ofloxacin	4–8	4–8	4–16	8–16	32
Nalidixic acid	8–16	8–16	8	8–16	>64
Aztreonam	0.5–4	0.25–2	0.25–0.5	0.5–1	2
Cefotaxime	0.25–4	0.25–2	1	1	2
Ceftriaxone	0.25–2	0.25–1	1	1	2
Ceftazidime	0.25–2	0.25–2	1	1	0.5
Imipenem	2–4	2–8	2–8	4–8	8
Piperacillin	0.25–2	0.5–1	1	1	2
Amikacin	0.25–1	0.25–1	0.25–1	0.5–1	0.25
Gentamicin	0.5–1	0.25–1	0.25–1	0.5–1	0.5
Chloramphenicol	1	1	4–16	4–8	1
Trimethoprim	1	1	4–8	8–16	1

[a] Compared with MICs of strains before exposure.
[b] Values in parentheses show the number of strains tested.

not observed in our experimental *E. cloacae* infection treated with β-lactam compounds (6a) when a sort of therapeutic index (i.e., antibiotic concentration in the peritoneal fluid over the no-growth concentration on the gradient plate) could be proposed.

A simpler interpretation can be advanced for explaining another difference between pefloxacin and ciprofloxacin. Administration of a single dose of 25 or 200 mg/kg of the former generated a similar resistance rate, whereas the resistance rate produced by the latter was dose dependent (Table 3). It is likely that the two dosages of pefloxacin both remained ineffective in influencing the resistance rate, whereas the higher dosage of ciprofloxacin was capable of inhibiting resistant variants which were not achieved by the lower dosage.

The influence of the dosing can also be seen in the multiple-dose therapies. Several 25-mg/kg doses of ciprofloxacin produced significantly less resistance in the 8-h schedule than in the 12-h schedule; in this respect, efficiency can also be gained with doses of 50 mg/kg. Here the lower resistance rates were associated with lower bacterial counts in the peritoneal cavity (Table 4, column 3), indicating that higher repeated doses or shorter dosing intervals ensured greater bacterial killing or more efficiently hampered bacterial regrowth.

TABLE 7. Pharmacokinetic data of pefloxacin and ciprofloxacin in mice

Antibiotic	Dosage (mg/kg)	Time after injection (min)	Antibiotic concn (μg/ml)[a] in:		Apparent $t_{1/2}$ (min) in peritoneal fluid
			Blood	Peritoneal fluid	
Pefloxacin	200	60	33.0	4.5	ND[b]
	25	30	19.5	8.9	100
		60	6.4	3.9	
		120	3.4	2.6	
		180	2.5	1.7	
Ciprofloxacin	25	30	5.6	5.7	105
		60	3.7	4.5	
		180	2.4	2.2	
Ceftazidime	50	60	23.2	57.8	ND
Piperacillin	50	60	6.8	5.7	ND

[a] Mean of three to six mice.
[b] ND, Not determined.

The PT_1 strains were often characterized by a relatively small decrease in their susceptibility to the quinolones. Also, compared with the parent strains, they produced lower bacterial counts in the peritoneal fluid of the control animals, perhaps because of a lower growth rate or a greater susceptibility to the host defenses. However, most of the treated animals challenged with the PT_1 strains (Table 5) died before dose 5 of quinolone, and the peritoneal bacterial counts were higher by several logs than those from the corresponding mice challenged with the parent strains (Table 4). This indicates that the PT_1 strains kept at least part of their virulence and that the quinolone therapy was less efficient against them.

The highly resistant PT_2 strain did not appear during a first treatment in animals infected with the parent *P. aeruginosa* but only after subinoculation of a PT_1 strain and a second therapeutic exposure. Unequal inoculum size of resistant cells in the two sets of experiments (only a few PT_1 cells in the first mouse and about 10^8 PT_1 cells in the rechallenged mouse) probably accounted for the postponed occurrence of the PT_2 strain.

The expression of resistance to the fluoroquinolones in members of the family *Enterobacteriaceae* can result from at least two mechanisms (5). Some bacteria possess an altered outer membrane permeability (4, 5) which typically produce four- to eightfold increases of the quinolone MICs and often, a decreased susceptibility to structurally unrelated antibiotics (5, 13). A second mechanism of resistance involves an altered A subunit of the DNA gyrase, providing higher increases of the quinolone MICs with no other changes of the antibiotic susceptibility patterns (5). Mechanisms of resistance were not investigated in the present study. However, by analogy with the studies mentioned above, the PT_1 strains are likely to have permeability changes, since they showed a decreased susceptibility to imipenem after their selection by a quinolone therapy. Recently, emergence of resistance to imipenem during imipenem therapy in clinical isolates of *P. aeruginosa* has been associated with diminished permeability of the bacterial outer membrane (10). In addition, some PT_1 isolates showed a decreased susceptibility to trimethoprim and chloramphenicol, the activity of

which can be affected by alterations of the outer membrane in *Enterobacteriaceae* (3, 5). However, contrary to the findings by other researchers (13), the activity of aminoglycosides and β-lactam compounds other than imipenem remained unaffected. The slightly greater susceptibility to aminoglycoside, if not due to experimental errors, remains unexplained. As for the unique PT_2 isolate, the decreased susceptibility to imipenem suggests that permeability changes of the originating PT_1 was retained, but the higher level of resistance to the quinolones probably involved an additional mechanism, in all likelihood an altered DNA gyrase.

In confirmation of previous studies (7, 9), combined therapy limited the emergence of resistance. The most efficient partner of the quinolones in this respect was ceftazidime, followed by amikacin. Piperacillin was not able to reduce the resistance rate of pefloxacin or ciprofloxacin. The relatively low piperacillin concentrations obtained in the peritoneal fluid (Table 7) after the 50-mg/kg dose used in our experiments probably accounted for this failure.

Some of our observations might be useful for the clinician. During therapy with pefloxacin or ciprofloxacin, resistance can emerge rapidly. The likelihood of such emergence is greater in infections when a large number of bacteria is expected or when a foreign body is present but appears to be influenced by the dosing. This concept obviously deserves further investigation but, at this point, our findings support the idea of avoiding underdosing of patients, especially at initiation of therapy, when the bacterial populations are numerous. Combination therapy may limit the emergence of resistance, but all combinations are probably not similar in this respect.

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