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Bactericidal Activities of Five Quinolones for *Escherichia coli* Strains with Mutations in Genes Encoding the SOS Response or Cell Division

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The bactericidal effects of five quinolones (at the optimum bactericidal concentration for strain AB1157) on 15 strains of *Escherichia coli* with mutations in genes for the SOS response or cell division was studied by a viable-count method. The kill rate data were normalized for growth rate and compared to those for the wild type, AB1157. Similar MICs of enoxacin and fleroxacin were obtained for all mutants; however, different mutants had differing susceptibilities to ciprofloxacin, norfloxacin, and nalidixic acid. Killing kinetic studies showed that mutants with constitutive RecA expression (*recA730* and *spr-55* mutants) survived longer than AB1157 with all quinolones. Mutants deficient in SOS induction, e.g., *recA430* and *lexA3* mutants, also survived longer, suggesting that induction of the SOS response by quinolones is harmful to wild-type cells. Recombination repair-deficient mutants (*recB21*, *recC22*, and *recD1009* mutants) were killed more rapidly than AB1157, as were excision repair mutants, except with nalidixic acid. Mutants which were unable to filament (*sfiA11* and *sfiB114* mutants) survived longer than AB1157 with all agents, but a mutant defective in the Lon protease was killed more quickly. It was concluded that (i) recombination and excision repair were involved in the repair of quinolone-damaged DNA and (ii) continuous induction (in response to exposure to quinolones) of the SOS response, and hence induction of the cell division inhibitor SfiA, causes cell filamentation and thereby contributes to the bactericidal activity of quinolones.

The bactericidal mechanism of quinolones is unclear; however, it has been proposed that the initial event is the inhibition of DNA synthesis (5, 35) by interference with the nick-sealing activity of DNA gyrase (9, 14, 29). In 1979, Kreuzer and Cozzarelli (20) suggested that the binding of a quinolone to DNA gyrase may cause it to act as a "cellular poison." It was suggested that nalidixic acid bound to DNA gyrase and DNA, thus trapping an enzyme intermediate at the replication fork, inhibiting the unwinding of the DNA duplex and hence DNA replication (7). Shen and Pernet (35) suggested that to form the complex, quinolones interact with DNA or a DNA gyrase-DNA complex (34).

There is excellent correlation between the concentration of quinolones required to inhibit DNA synthesis in *Escherichia coli* by 50% and the MIC (5, 31). However, the inhibition of protein synthesis by chloramphenicol decreases the bactericidal activity of quinolones (36) and inhibits induction of RecA (32) but has no effect on the inhibition of DNA synthesis (7, 31). Therefore, it has been proposed that the primary event in the bactericidal action of quinolones is the inhibition of DNA synthesis due to the interaction with DNA gyrase; induction of the SOS response is consequential but also contributes to the bactericidal activity of these agents (31).

Nalidixic acid and UV light have been shown to induce RecA, the SOS regulatory protein in *E. coli* (13, 18) and other members of the family *Enterobacteriaceae* (40). It has also been demonstrated that quinolones induce RecA and other SOS response gene products in *E. coli* (30–32, 37) and in quinolone-susceptible and -resistant mutants of members of the *Enterobacteriaceae* (40). There is excellent correlation between the concentration of a quinolone causing maximum RecA induction, the optimum bactericidal concentration (OBC), and the MIC (30, 31, 37). At concentrations above the OBC and the maximum RecA-inducing concentration, quinolones inhibit protein synthesis, which is required for expression of SOS genes.

The physiological consequences of SOS induction include the inhibition of cell division and enhanced DNA repair (38). Induction of the SOS response may enhance the survival of a damaged cell because of increased DNA repair (2, 4, 19), or RecA may protect gapped DNA from attack by RecBC exonuclease (11). It has also been suggested that induction of the SOS response may be involved in the bactericidal mechanism of quinolones (26, 30–32). The inhibitory effect of quinolones on DNA gyrase may result in the decreased ability of a cell to repair its damaged DNA, or continuous cell filamentation per se may be lethal to the cell.

Previous studies done in our laboratory examined the bactericidal effect of quinolone concentration on SOS mutants of *E. coli* (41). The data suggested that individual quinolones differed in precise details in their effects on different mutants. Induction of the SOS response in wild-type strains treated with fleroxacin and enoxacin appeared to enhance cell survival, whereas the effect of ciprofloxacin or nalidixic acid on the mutants was difficult to interpret with respect to their SOS phenotype. A recombination-deficient mutant, AB2470 (*recB21*), was hypersusceptible to fluoroquinolones, and a cell division mutant, N1497 (*sfiB*), showed increased survival in the presence of all quinolones studied.

Lewin et al. (21) studied the effect of 50 μg of nalidixic acid per ml on strains of *E. coli* lacking an inducible SOS response. *E. coli* strains with *recA13* and *recB21* mutations, which lack SOS induction and recombination repair, were hypersusceptible to nalidixic acid. Recombination-profi-

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TABLE 1. Mutants of *E. coli* defective in components of the SOS response and cell division

Strain	Genotype	Description	Source	Reference
AB1157	F− *thr leu pro his thi argE lacY gal ara mtl xyl tsx supE* λ *rpsL*	Wild type	S. Casaregola	
SC30	AB1157 (DM2001) *recA730 spr tif sfiA*	Constitutive production of proteolytic RecA, constitutive SOS induction	S. Casaregola	42
GY2831	AB1157 *recA430*	Incomplete SOS induction due to defective RecA protease	S. Casaregola	8
SC23	AB1157 (DM2001) *spr-55 spr tif sfiA*	Constitutive SOS induction due to amber mutation in *lexA*	S. Casaregola	28
DM49	AB1157 *lexA*	SOS induction deficient, recombinational repair proficient	R. G. Lloyd	25
LG372	AB1157 *recA-C*	Constitutive RecA expression due to *recA* operator mutation	S. Casaregola	32
JM18	AB1157 *recA718 uvr*	Recombinational repair proficient, excision repair deficient	S. Casaregola	42
AB2470	AB1157 *recB21*	SOS induction deficient, recombinational repair deficient, lacks exonuclease V	S. Casaregola	12
JC5489	AB1157 *recC22*	SOS induction deficient, recombinational repair deficient	R. G. Lloyd	12
N2525	AB1157 *recD1009*	Recombinational repair deficient	R. G. Lloyd	22
AB1886	AB1157, *uvrAb* deficient	Excision repair deficient	R. G. Lloyd	1
AB1885	AB1157, *uvrB* deficient	Excision repair deficient	R. G. Lloyd	1
GC2277	AB1157 *sfiA11*	Deficient in SOS-induced filamentation, insertion mutation in *sfiA*	G. R. Drapeau	3
N1497	DM961 (*leu*$^+$ *sfiB Hfr* derivative of AB1157 × GC895 [Hfrase]) *sfiB114*	Defective cell division protein, no interaction with SfiA	R. G. Lloyd	15
GD40	AB1157 *tsM1*		G. R. Drapeau	3
AB1899	AB1157 *lon*	Deficient in Lon protease, decreased degradation of SfiA	R. G. Lloyd	6

cient, SOS-deficient mutants with *lexA3* and *recA430* mutations showed a pattern of cell death similar to that of the parent strain. It was concluded that induction of the SOS response by nalidixic acid did not enhance killing or protect the cells from quinolone action, but it was suggested that recombination repair was involved in the repair of quinolone-induced DNA lesions.

In this study, the bactericidal action of five quinolones on *E. coli* AB1157 and derived mutants defective in genes encoding the SOS response or cell division was investigated by performing killing kinetic studies at the OBC of AB1157 to confirm or eliminate the role of the SOS response in the bactericidal action of quinolones.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Table 1 lists the bacterial strains used in the study. All were either isogenic to *E. coli* AB1157 or derivatives of this strain. Bacteria were grown in sterile minimal broth Davis without dextrose (Difco), supplemented with 10 μg of thiamine hydrochloride (Sigma) per ml, 0.4% Casamino Acids (Difco), and 0.2% D-glucose (BDH) and cultured on IsoSensitest agar (Unipath) at 37°C.

Antibiotics and determination of susceptibility. Fleroxacin (Roche), nalidixic acid (Sterling-Winthrop), enoxacin and chloramphenicol (Warner-Lambert/Parke-Davis), ciprofloxacin (Bayer AG), norfloxacin and cefoxitin (Merck Sharp & Dohme), and tetracycline (Lederle) were obtained as gifts and made up and used according to the manufacturers' instructions. The MIC of each antibiotic for each strain was determined by a standard agar doubling dilution method. An inoculum of 10^6 CFU per spot was applied to the agar by a multipoint inoculator (Denley-Tech) delivering 1 μl per spot. The MIC was defined as the lowest concentration at which no more than two colonies were detected after aerobic incubation at 37°C for 24 h. A slight haze of growth was ignored.

Determination of the OBC. The OBC of each quinolone for AB1157 was determined essentially as described by Walters and Piddock (40). Briefly, 100 ml of broth was inoculated with 2 ml of an overnight culture and incubated at 37°C with shaking, until the culture was in the exponential phase and the viable-cell count was 2×10^8 CFU/ml. Preliminary experiments were performed to confirm that at this viable-cell count all strains were growing exponentially. Ten milliliters of culture was withdrawn and added to bottles containing quinolones, and incubation was continued for 1 h. The culture was harvested by centrifugation, washed with phosphate-buffered saline (PBS), and serially diluted in 10 ml of PBS. One hundred microliters of the appropriate dilutions was spread onto agar plates and incubated overnight at 37°C, and the viable-cell count was determined. The effect of protein synthesis on quinolone killing was studied by the addition of 20 μg of chloramphenicol per ml simultaneously with the quinolone.

Determination of growth kinetics. One hundred milliliters of broth was inoculated with 2 ml of an overnight culture and incubated at 37°C with shaking. At timed intervals, samples were taken, the A_{675} was determined, and 100 μl of the sample was serially diluted and the number of CFU per milliliter was determined as described above. The growth of all strains was measured, and the data were plotted graphically. The generation time was calculated from the linear section of the growth curve by the equation generation time (exponential growth rate) = $(\log b - \log a)/[(t_2 - t_1) \times \log_{10}2]$, where t_1 is the time (in hours) at the start of linear growth, t_2 is the time (in hours) at the start of the linear

TABLE 2. Antimicrobial susceptibilities of strains used in this study at 10^6 CFU per spot on agar

Strain	Genotype	MIC (μg/ml) of[a]:							
		NAL	CIP	NOR	ENX	FLX	FOX	CHLOR	TET
AB1157	Wild type	4	0.03	0.016	0.12	0.12	2	2	2
SC30	*recA730*	4	0.016	0.03	0.12	0.06	2	2	2
GY2831	*recA430*	16	0.03	0.03	0.12	0.12	4	8	1
SC23	*spr-55*	8	0.016	0.03	0.12	0.12	2	2	2
DM49	*lexA3*	16	0.016	0.03	0.06	0.12	8	8	4
LG372	*recAO*^{c}	2	0.25	0.016	0.12	0.06	4	1	1
JM18	*recA718*	8	0.25	0.03	0.25	0.25	8	2	2
AB2470	*recB21*	4	<0.008	<0.03	0.12	0.06	8	4	8
JC5489	*recC22*	8	<0.008	0.03	0.25	0.5	4	4	2
N2525	*recD1009*	8	0.03	0.25	0.25	0.25	8	4	4
AB1886	*uvrA6*	16	0.016	0.06	0.12	0.25	4	4	2
AB1885	*uvrB301*	32	0.03	0.06	0.12	0.12	4	2	2
GC2277	*sfiA11*	8	0.03	0.12	0.25	0.12	8	8	4
N1497	*sfiB114*	32	0.03	0.06	0.12	0.12	8	8	4
GD40	*tsM1*	16	0.03	0.12	0.25	0.25	8	8	4
AB1899	*lon*	16	0.06	0.06	0.25	0.25	8	8	8

[a] NAL, nalidixic acid; CIP, ciprofloxacin; NOR, norfloxacin; ENX, enoxacin; FLX, fleroxacin; FOX, cefoxitin; CHLOR, chloramphenicol; TET, tetracycline.

growth, a is the viable-cell count at t_1, and b is the viable-cell count at t_2.

Determination of the kinetics of quinolone killing. Two milliliters of an overnight culture was added to 100 ml of broth and incubated until the viable-cell count was 2×10^8 CFU/ml. At time zero, 10 ml was withdrawn and a quinolone was added to the remaining culture to the concentration equivalent to the OBC of that agent for AB1157. At timed intervals, 10-ml samples were withdrawn and the viable-cell counts were determined.

The rate of killing (kill time) in the presence of each quinolone at the OBC was calculated by the equation described for the calculation of generation time, except that t_1 was the time (in hours) at the start of linear cell death and t_2 was the time (in hours) at the end of linear cell death. For each strain and each agent, the kill time value was normalized to the growth rate value and multiplied by -1 to enable comparisons between strains to be made. The standard deviation of the mean of normalized values from replicate experiments with each strain and each antibiotic was calculated.

RESULTS

Antibiotic susceptibility. DM49 (*lexA3*), AB1886 (*uvrA6*), AB1899 (*lon*), GD40 (*tsM1*), and GY2831 (*recA430*) had a fourfold increase in the MIC of nalidixic acid compared with the wild-type strain, AB1157 (Table 2). AB1885 (*uvrB301*) and N1497 (*sfiB114*) were eightfold less susceptible to nalidixic acid. The MICs of ciprofloxacin for JM18 (*recA718*) and LG372 (*recAO*^{c}) were fourfold higher than that for the wild-type strain. AB2470 (*recB21*) and JC5489 (*recC22*) were hypersusceptible, with an MIC of ciprofloxacin of ≤0.008 μg/ml. The MIC of norfloxacin was increased fourfold for AB1886 (*uvrA6*), AB1885 (*uvrB301*), N1497 (*sfiB114*), and AB1899 (*lon*) and eightfold for GD40 (*tsM1*) and GC2277 (*sfiA11*) compared with that for AB1157. All the mutants had susceptibilities to enoxacin and fleroxacin similar (plus or minus one dilution step) to that of the wild type. DM49 (*lexA3*), JM18 (*recA718*), AB2470 (*recB21*), N2525 (*recD1009*), N1497 (*sfiB114*), GD40 (*tsM1*), and GC2277 (*sfiA11*) were fourfold less susceptible to cefoxitin than AB1157. DM49, N1497, GD40, GC2277, and GY2831 were also fourfold less susceptible to chloramphenicol, and the MIC of tetracycline for AB2470 (*recB21*) was also raised fourfold. AB1899 (*lon*) was fourfold less susceptible than the wild type to all three chemically unrelated agents.

Killing kinetics. After 3 h of incubation, the OBC of nalidixic acid for AB1157 was 75 μg/ml and that of ciprofloxacin was 2 μg/ml. Enoxacin, fleroxacin, and norfloxacin had OBCs of 7 μg/ml for AB1157 after 1 h of incubation. An incubation time of 1 h as opposed to 3 h did not affect the OBC (data not shown).

The killing curves of all quinolones and all mutants were biphasic; an initial rapid linear phase (from which the kill rate was calculated) was followed by a slower phase during which the kill rate decreased. A short lag phase was also observed with nalidixic acid and some of the mutants before the onset of rapid bactericidal activity. Table 3 shows whether the normalized kill rate of each quinolone was similar to, lower than, or higher than that of the wild-type strain, AB1157. The mean generation time of AB1157 from four experiments was 1.76 generations per h, with a standard deviation of 0.217 generations per h.

Except for JM18 (*recA718*), strains with mutations in *recA* or *lexA* were killed more slowly (50% of the strains and antibiotics) than or at a rate (43% of the strains and antibiotics) similar to that of AB1157 by all quinolones examined (Table 3; Fig. 1). Five strains (JM18, SC30, SC23, LG372, and DM49) had higher growth rates than AB1157 (3.8, 3.15, 2.78, 2.29, and 4.3 generations per h, respectively). GY2831 (*recA430*) had a growth rate similar to that of AB1157 but was killed more slowly than AB1157 by three quinolones. DM49 varied in its responses to the antibiotics, with no definite pattern, unlike the other strains.

The exponential growth rates of AB2470, JC5489, and N2525 (*recB21*, *recC22*, and *recD1009*, respectively) were similar to that of the wild-type strain (2.04, 1.95, and 1.84 generations per h, respectively), although the lag phases of AB2470 and N2525 were longer (data not shown). All were killed more rapidly than AB1157 by all quinolones examined.

The growth rates of AB1886 (*uvrA6*) and AB1885 (*uvrB301*) were similar to that of the wild type (2.06 and 1.85 generations per h, respectively). Both mutants were killed more rapidly than AB1157 by the fluoroquinolones (Fig. 2), but their rates of cell death with nalidixic acid (1.33 and 2.31

TABLE 3. Killing rates of five quinolones for SOS and cell division mutants and AB1157

Genes or functions mutated	Strain	Genotype	Killing rate (generations/h) for[a]:				
			Nalidixic acid	Ciprofloxacin	Enoxacin	Fleroxacin	Norfloxacin
None	AB1157	Wild type	4.35 ± 1.2	11.09 ± 2.6	5.32 ± 1.8	5.98 ± 1.9	7.96 ± 2.6
recA and *lexA*	SC30	*recA730*	0.28 (−)	1.96 (−)	1.03 (−)	0.43 (−)	0.03 (−)
	GY2831	*recA430*	2.82 ± 0.6 (−)	4.5 ± 2.7 (−)	5.8 (=)	7.26 ± 1.3 (=)	6.1 (−)
	SC23	*spr-55*	2.17 ± 0.3 (−)	6.99 ± 1.2 (−)	4.11 ± 0.5 (=)	5.9 ± 0.5 (=)	6.71 ± 1.3 (=)
	DM49	*lexA3*	0.97 ± 0.3 (	5.13 (−)	4.2 (=)	1.14 (−)	4.9 (−)
	LG372	*recAO*^{c}	3.12 (=)	8.68 (=)	5.78 (=)	4.65 ± 1.6 (=)	7.33 ± 1.9 (=)
	JM18	*recA718*	3.21 (=)	5.21 (−)	9.51 (+)	7.17 (+)	8.41 ± 1.5 (=)
Recombination and repair	AB2470	*recB21*	8.82 (+)	15.44 (+)	10.33 (+)	13.66 (+)	15.44 (+)
	JC5489	*recC22*	7.00 (+)	NC (+)	9.64 ± 4.5 (+)	11.63 (+)	NC (+)
	N2525	*recD1009*	8.46 (+)	NC (+)	7.95 (+)	11.79 (+)	7.05 (+)
Excision repair	AB1886	*uvrA6*	1.33 (−)	14.6 (+)	6.79 ± 0.4 (+)	8.27 (+)	7.75 (=)
	AB1885	*uvrB301*	2.31 ± 0.05 (−)	13.36 (+)	9.36 (+)	10.75 (+)	11.57 (+)
Cell division	GC2277	*sfiA11*	1.35 (−)	9.31 (−)	1.13 (−)	5.07 ± 0.6 (−)	4.31 ± 1.7 (−)
	N1697	*sfiB114*	3.8 (−)	9.52 (−)	2.19 ± 1.4 (−)	4.26 ± 1 (−)	4.61 (−)
	GD40	*tsM1*	1.43 (−)	8.0 (−)	4.23 ± 0.3 (−)	4.11 (−)	1.67 (−)
	AB1899	*lon*	2.97 ± 1.2 (−)	14.43 (−)	8.81 ± 4.4 (+)	9.48 (+)	13.16 (+)

[a] Mean ± standard deviation is shown for experiments performed three or more times. All other data are the means of two experiments. Also indicated is whether the kill rate was the same (=), enhanced (+), or decreased (−) relative to that of the wild type. Some strains were killed so rapidly that by the first sampling time (30 min), there were no detectable bacteria; therefore, the rate was not calculable (NC).

generations per h, respectively) were lower than that of AB1157.

The kinetics of quinolone killing was also studied with strains with mutations in cell division genes. GC2277 (*sfiA11*) and N1497 (*sfiB114*) had growth rates similar to that of AB1157 but were killed more slowly by all quinolones examined. AB1899 (*lon*) had a lower growth rate and GD40 (*tsM1*) had a higher growth rate than the wild-type strain. The fluoroquinolones had higher kill rates for AB1899 (*lon*) than for AB1157; however, nalidixic acid killed this mutant more slowly than AB1157. GD40 (*tsM1*) was killed more slowly than AB1157 by all agents tested.

McDaniel and colleagues (26) and Lewin et al. (21) have studied the effect of nalidixic acid on mutants of *E. coli* with mutations in *lexA*, *uvrB*, and *recA* (*recA430*). The concentrations used by McDaniel et al (26) and Lewin et al. (21) were 100 and 50 μg/ml, respectively, which differ from that used in this study. Therefore, experiments were performed in parallel to compare the effects of 50, 75, and 100 μg of nalidixic acid per ml on the kinetics of death of AB1157 and mutants DM49 (*lexA3*), AB1885 (*uvrB301*), and GY2831 (*recA430*). The effect of 50 or 100 μg of nalidixic acid per ml on the mutants did not differ from the effect of 75 μg/ml (Table 4).

The simultaneous addition of chloramphenicol with a quinolone decreased quinolone killing for AB1157 and for the three mutants studied, GY2831 (*recA430*) and SC30 (*recA730*) (Fig. 3) and LG372 (*recAO*^{c}) (data not shown).

DISCUSSION

The MICs of enoxacin and fleroxacin were essentially the same for the mutants and AB1157, whereas the data for ciprofloxacin and nalidixic acid show some differences in the susceptibilities of the mutants. AB2470 (*recB21*) was hypersusceptible to ciprofloxacin, which was anticipated, as similar observations had been previously reported by Piddock and Wise (32) and Lewin et al. (21). The cell division mutant N1497 (*sfiB114*), which lacks the ability to filament, is resistant to nalidixic acid (MIC, 32 μg/ml). This observation

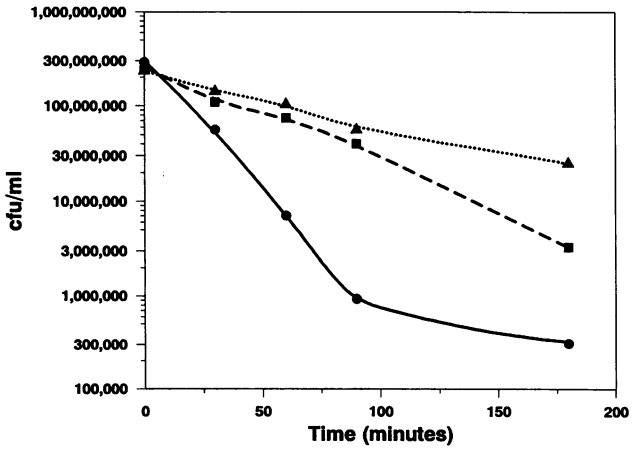

FIG. 1. Effect of 75 μg of nalidixic acid per ml on SC30 (*recA730*) (■), GC2277 (*sfiA11*) (▲), and AB1157 (●).

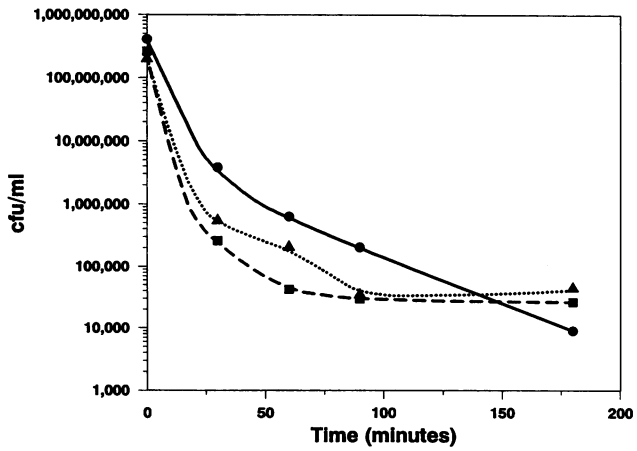

FIG. 2. Effect of 7 μg of fleroxacin per ml on AB1886 (*uvrA6*) (■), AB1885 (*uvrB301*) (▲), and AB1157 (●).

TABLE 4. Effect of quinolone concentration on the rate of killing of selected mutants of *E. coli*

Strain	Genotype	Killing rate with nalidixic acid concn (μg/ml) of[a]:		
		50	75	100
AB1157	Wild type	6.28 (3.56)	6.57 (3.73)	8.0 (4.54)
AB1885	*uvrB301*		3.3 (1.78)	4.2 (2.27)
DM49	*lexA3*	2.42 (0.56)	2.83 (0.65)	
GY2831	*recA430*	3.78 (1.69)	4.23 (1.89)	

[a] Killing rates are given in generations per hour. Results in parentheses are normalized for growth rate.

suggests that cell filamentation is important in the mechanism of killing by nalidixic acid. Differences that were observed between strains and their responses to quinolones in the assessment of killing kinetics were presumably due to the fact that susceptibility was measured after 24 h of exposure to the test agent, and killing kinetics are determined with exponentially growing bacteria over a period of 8 h. MICs of chemically unrelated agents that were higher for mutants than for AB1157 were presumably due to the pleiotropic effects on cellular metabolism that some of the mutations confer.

In the killing kinetic experiments, it was assumed that the rate of killing was a linear function of the growth rate (as with β-lactams), so all killing kinetic data (taken from the linear portion of the curve) were normalized by division by the growth rate. The OBC for AB1157 was chosen as the quinolone concentration to use in all experiments, since in a previous study of five quinolones the response of some mutants was quinolone and concentration dependent (41). Therefore, for easy and direct comparison, the concentration that resulted in maximal killing of the progenitor strain, AB1157, was used throughout.

Analysis of the killing rate data showed that four mutants with mutations affecting RecA expression, SC30 (*recA730*), SC23 (*spr-55*), GY2831 (*recA430*), and DM49 (*lexA3*), survived longer in the presence of most quinolones than

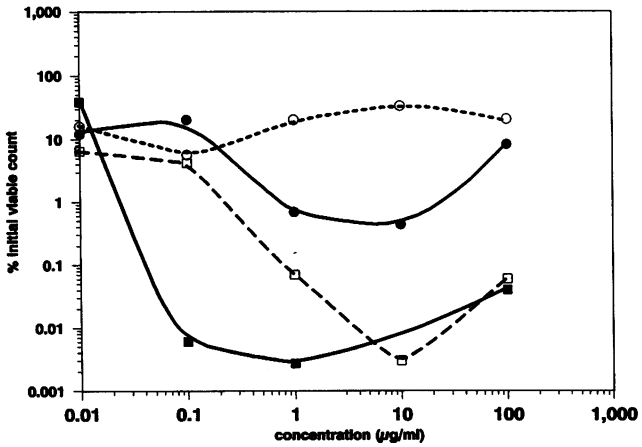

FIG. 3. Effect of chloramphenicol (20 μg/ml) on the dose response of SC30 (*recA730*) and GY2831 (*recA430*) to quinolones. Symbols: ●, SC30 with ciprofloxacin but without chloramphenicol; ○, SC30 with ciprofloxacin and chloramphenicol; ■, GY2831 with fleroxacin but without chloramphenicol; □, GY2831 with fleroxacin and chloramphenicol.

AB1157. It has been suggested that constitutive RecA expression enhances cell survival in the presence of a quinolone because the RecA protein may protect damaged DNA from attack by RecBC exonuclease (11). Alternatively, the SOS response may contribute to the repair of quinolone-induced DNA damage. SC30 has also been described as having increased resistance to UV light (42). Both SC30 and SC23 have functional recombinational repair activity and would be expected to have induced levels of SOS repair (38, 42), suggesting that the enhanced survival is due to the increased repair of quinolone-induced DNA lesions by recombinational and excision repair. SOS induction in GY2831 is less efficient than in AB1157, as the RecA protein has a reduced ability to cleave LexA, and it has been suggested that a greater amount of inducing signal is required to achieve the same level of SOS induction as in AB1157 (38). However, like those of DM49, the recombination properties of GY2831 are unimpaired (8). A previous study in our laboratory (41) with GY2831 also showed increased survival in the presence of quinolones, although Lewin et al. (21) reported equal susceptibility of *E. coli* SC1657 (*recA430*) and its parent strain, SC1656, to 50 μg of nalidixic acid per ml. In the present study, the concentration of nalidixic acid did not affect the data; therefore, the discrepancy between the two sets of data is probably due to the different genetic backgrounds of the mutants. The strains used by Lewin et al. (21) both contained an additional mutation in *sfiB*, a cell division locus, which may affect survival in the presence of quinolones. GY2831 does not contain a mutation in *sfiB*, and therefore the observed difference between GY2831 and AB1157 is due to the mutation in *recA* alone. The discrepancy between the reproducible data obtained with DM49 and those of Lewin et al. (21) with the same strain and nalidixic acid cannot be explained.

Strain LG372 (*recAO*^{c}) was killed at a rate similar to that of AB1157 by all agents. The RecA protein produced by LG372 is functional in recombinational repair but is thought to be proteolytically inactive, requiring an SOS-inducing signal for activation (11). These data suggest that proteolytically active RecA is important in resisting quinolone action.

SC23 also has constitutive expression of other SOS response genes, including the cell division inhibitor *sfiA*. The *lexA*(Def) mutation alone would be lethal because of continuous cell filamentation, but the presence of a mutation in *sfiA* prevents the inhibition of division (10, 28). Therefore, the increased survival of SC23 may be due to the inability of the bacterial cells to inhibit cell division.

Strain JM18 has a partial deficiency in recombinational and excision repair, so SOS induction may be suboptimal or RecA718 may have structural changes compared with RecA (42). This strain had a variable response to quinolones which could not be correlated to the phenotype or genotype.

The hypersusceptibility of AB2470 (*recB21*), JC5489 (*recC22*), and N2525 (*recD1009*) to quinolones confirmed the earlier observations that recombinational repair is required for the repair of quinolone-induced DNA damage (21, 41). In addition, strains with mutations affecting excision repair, AB1886 (*uvrA6*) and AB1885 (*uvrB301*), were also killed more rapidly by the fluoroquinolones, but not by nalidixic acid, than AB1157. The observations with nalidixic acid and the *uvr* mutants do not agree with the data of McDaniel et al. (26), which showed that a *uvrB* mutant was killed more rapidly by nalidixic acid than the parent strain, AB1157. However, the data from the present study do support the proposal of Lewin et al. (21) that SOS-dependent excision repair is not involved in the repair of lesions caused by

nalidixic acid. Expression of *uvrA* is inducible by SOS-inducing agents and regulated by RecA (19); hence, excision repair is SOS dependent, which suggests that SOS induction enhances DNA repair of damage caused by fluoroquinolones. Kenyon and Walker (19) proposed that the UvrAB endonuclease repaired DNA lesions or protected the DNA from damage.

In wild-type cells, SfiA is induced after treatments which induce the SOS response (16). It interacts reversibly with the cell division protein FtsZ (24), causing the inhibition of cell division. Two strains with mutations inhibiting the binding between SfiA (GC2277 [*sfiA11*]) and SfiB (FtsZ [23] and N1497 [*sfiB114*] [15]) survived longer in the presence of quinolones than AB1157, suggesting that the inhibition of cell division by quinolones in the wild-type strain is a lethal event. In addition, GD40 (*tsM1*) also had increased survival in the presence of the fluoroquinolones. The role of the wild-type *ts*M protein in the control of cell division is not completely understood; this protein may either inhibit the activity of FtsZ or be an activator of FtsZ (3). GD40 and AB1899 (*lon*) share certain properties, e.g., the formation of mucoid colonies, decreased lysogenation of lambda and P1 phages, and reduced survival after UV treatment when plated on rich medium compared with a minimal medium (6, 39), but the genetic regulation of the phenotype is different. AB1899 and GD40 differed in their response to fluoroquinolones, although both mutants had increased survival with nalidixic acid compared with the wild type. The mutant Lon protein has a decreased ability to degrade SfiA (27, 33), and AB1899 was killed more quickly by quinolones, presumably as more SfiA was available to inhibit cell division (shown by hyperfilamentation of this mutant).

The different response of three mutants to nalidixic acid and to fluoroquinolones suggests that although the data from this study have suggested that fluoroquinolones have a common mechanism of action, this mechanism may be different from or supplementary to that of the older quinolone analog. Alternatively, the use of a minimal salts growth medium may affect the susceptibility of some mutants to SOS-inducing agents, as suggested for UV light by Walker et al. (39) and Drapeau et al. (6).

Analysis of the killing kinetic data by relating the rate of killing to the growth rate of the mutants showed that, with the exception of three mutants in the presence of nalidixic acid, all quinolones with all mutants produced the same pattern of cell death. The conclusions drawn from this analysis propose that in wild-type cells (i) recombinational repair and the induction of the excision repair enzyme UvrAB endonuclease repair quinolone-induced DNA damage and (ii) the continuous induction of the cell division inhibitor SfiA in the presence of a quinolone causes cell filamentation, which contributes to quinolone killing. Induction of the SOS response therefore contributes to the bactericidal effect of quinolones, because induction and activation of RecA are required for cleavage of the LexA repressor and hence the derepression of other SOS response genes, including the cell division inhibitor SfiA. SfiA is induced by the SOS-inducing agents nalidixic acid and UV light (16), and Phillips and colleagues (30) demonstrated that quinolones also caused induction of *sfiA* at concentrations similar to the OBC described by Smith (36). The induction of RecA is inhibited by protein synthesis inhibitors (32). The contribution of SOS induction to the bactericidal activity of quinolones explains why the inhibition of protein synthesis by chloramphenicol antagonizes the action of quinolones (31). Decreased quinolone killing in the presence of chloramphenicol in SOS mutants suggests that induction of the SOS induction contributes only in part to the bactericidal activity of quinolones. This in turn suggests that the principal mechanism of action of quinolones is independent of protein synthesis and therefore must reside with an interaction with DNA gyrase.

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