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MINIREVIEW

Quinolone-Mediated Bacterial Death[▽]

Karl Drlica,[1]* Muhammad Malik,[1] Robert J. Kerns,[2] and Xilin Zhao[1]

Public Health Research Institute, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, 225 Warren St., Newark, New Jersey 07103,[1] and Division of Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, Iowa 52242[2]

The fluoroquinolones are broad-spectrum antibacterial agents that are becoming increasingly popular as bacterial resistance erodes the effectiveness of other agents (fluoroquinolone sales accounted for 18% of the antibacterial market in 2006) (41). One of the attractive features of the quinolones is their ability to kill bacteria rapidly, an ability that differs widely among the various derivatives. For example, quinolones differ in rate and extent of killing, in the need for aerobic metabolism to kill cells, and in the effect of protein synthesis inhibitors on quinolone lethality. Understanding the mechanisms underlying these differences could lead to new ways for identifying the most bactericidal quinolone derivatives.

Before describing the types of damage caused by the quinolones, it is useful to define lethal activity. Operationally, it is the ability of drug treatment to reduce the number of viable cells, usually measured as CFU on drug-free agar after treatment. This assay is distinct from measurements that detect inhibition of growth (e.g., MIC), since with the latter bacteria are exposed to drug throughout the measurement. The distinction between killing and blocking growth is important because it allows susceptibility determinations to be related to particular biological processes. For example, inhibition of growth is typically reversed by the removal of drug, while cell death is not. Thus, biochemical events associated with blocking growth should be readily reversible, while those responsible for cell death should be difficult to reverse. Reversibility can be used to distinguish among quinolone derivatives and assign functions to particular aspects of drug structure. Moreover, protective functions, such as repair and stress responses, can be distinguished by whether their absence affects inhibition of growth, killing, or both.

The intracellular targets of the quinolones are two DNA topoisomerases: gyrase and topoisomerase IV. Gyrase tends to be the primary target in gram-negative bacteria, while topoisomerase IV is preferentially inhibited by most quinolones in gram-positive organisms (28). Both enzymes use a double-strand DNA passage mechanism, and it is likely that quinolone biochemistry is similar for both. However, physiological differences between the enzymes exist, some of which may bear on quinolone lethality.

* Corresponding author. Mailing address: Public Health Research Institute, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, 225 Warren St., Newark, NJ 07103. Phone: (973) 854-3360. Fax: (973) 854-3101. E-mail: drlica@phri.org.
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In the present minireview we consider cell death through a two-part "poison" hypothesis in which the quinolones form reversible drug-topoisomerase-DNA complexes that subsequently lead to several types of irreversible (lethal) damage. Other consequences of quinolone treatment, such as depletion of gyrase and topoisomerase IV activity, are probably less immediate (42). To provide a framework for considering quinolone lethality, we begin by briefly describing the drug-topoisomerase-DNA complexes. Readers interested in a more comprehensive discussion of quinolones are referred to a previously published work (28).

QUINOLONE-TOPOISOMERASE-DNA COMPLEXES

As a normal part of their reaction mechanism, gyrase and topoisomerase IV introduce a pair of staggered, single-strand breaks (nicks) into DNA and become covalently bound to the 5′ ends of the cleaved DNA (55, 57). Quinolones bind rapidly to enzyme-DNA complexes (35), probably before DNA cleavage occurs (Fig. 1, step b_1); drug binding occurs with mutant gyrase (*gyrA*) or topoisomerase IV (*parC*) that fails to cleave DNA (9, 54). After drug binding, a slower, DNA cleavage-associated step occurs (35) that results in drug-mediated inhibition of religation of the DNA ends by topoisomerases (1). In a sense, quinolones trap the bacterial type II topoisomerases on DNA (17, 23, 73, 75) (Fig. 1, step b_2). The resulting structures have been termed cleavable, cleavage, and cleaved complexes. In the present study we refer to them as cleaved complexes to emphasize that phosphodiester bonds in the DNA moiety are broken (the term ternary complex is reserved for the early step in drug-enzyme-DNA complex formation in which the DNA is unbroken). A variety of quinolone-mediated phenomena follow from formation of cleaved complexes. To better understand lethal processes, we briefly describe key features of the DNA and protein components of the complexes. Since crystal structures have not been reported for cleaved complexes, tentative inferences concerning the relative positions of drug, protein, and DNA are drawn from biochemical experiments, partial structures of the bacterial topoisomerases, and complete structures of eukaryotic topoisomerase II.

Evidence for the DNA being cleaved derives from the recovery of DNA fragments when cleaved complexes are treated with protein denaturants, such as sodium dodecyl sulfate (SDS) (Fig. 1, step g). These DNA fragments are covalently bound to the GyrA or ParC proteins (55, 57). While cleaved complexes preferentially form at particular sites on DNA (44,

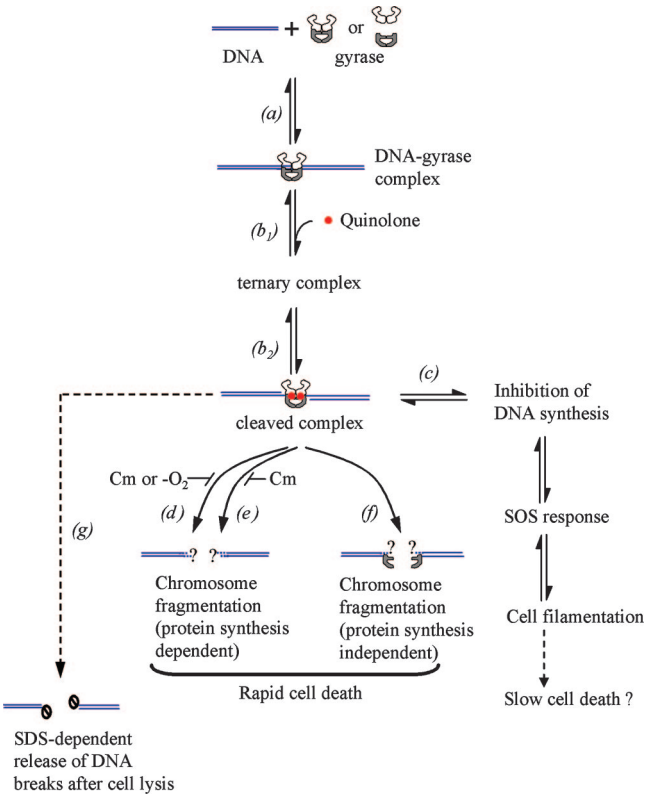

FIG. 1. Schematic representation of quinolone action with gyrase as the primary target. (Step a) Binding of gyrase to DNA. (Step b) Reversible formation of quinolone-gyrase-DNA complexes that rapidly block DNA replication. Step b_1 depicts binding of quinolone to gyrase-DNA complexes before DNA cleavage; step b_2 represents binding after DNA cleavage. (Step c) Inhibition of replication leads to induction of the SOS response and cell filamentation. (Step d) Lethal chromosome fragmentation that requires ongoing protein synthesis in aerobic conditions, as seen with nalidixic acid treatment of *E. coli*. (Step e) Lethal chromosome fragmentation that requires on-going protein synthesis but not aerobic conditions, as seen with norfloxacin treatment of *E. coli*. (Step f) Lethal chromosome fragmentation that requires neither ongoing protein synthesis nor aerobic conditions, as seen for PD161144 with *E. coli*. (Step g) DNA breakage detected after treatment of cell lysates with an ionic detergent such as SDS. Not shown are effects on transcription. Question marks indicate uncertainty about slow death and the nature of the DNA ends.

48, 60), the nucleotide sequence requirement is loose, and the fragment size is small relative to the whole chromosome (4, 7, 16, 73). Consequently, quinolone treatment creates many DNA-break-containing complexes throughout the chromosome.

Cleaved complex formation is reversed by the removal of quinolone, by the addition of the chelating agent EDTA, or by mild thermal treatment. In each case, the topoisomerase reseals the DNA nicks, and intact DNA is seen after treatment with SDS (17, 53, 64, 73, 75). Since chromosomal DNA supercoiling is not relaxed by bacteriostatic quinolone treatment (73) nor is plasmid DNA superhelicity altered when quinolone-gyrase complexes are reversed by EDTA (44), the DNA ends must be constrained in the complexes. The reversible nature of the cleaved complexes emphasizes that they are not by themselves lethal.

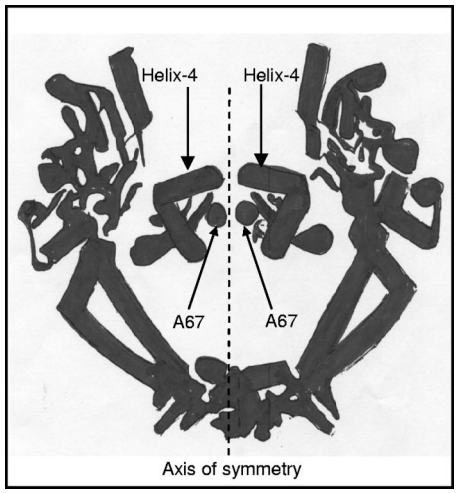

FIG. 2. Sketch of dimerized GyrA59 fragment with DNA and protein gates closed. GyrA-GyrA dimer interfaces are located along the axis of symmetry; arrows point to helix-4 of each GyrA subunit and to positions of *E. coli* amino acid number 67.

The location and conformation of DNA in the complexes is poorly defined. However, examination of quinolone binding suggests that gyrase and topoisomerase IV may perturb DNA structure (54), perhaps by forming a single-stranded bubble that facilitates quinolone binding (44). Preferential binding of quinolones to single-stranded DNA correlates with in vitro quinolone potency (i.e., the 50% inhibitory concentration), while binding to double-stranded DNA does not (59). These data are consistent with the observation that norfloxacin binding to negatively supercoiled DNA is saturable (70), since negative supercoils confer some single-stranded character. Binding of quinolone to circular, relaxed DNA occurs only when gyrase, which by itself binds quinolones poorly, is also present to form complexes (71). Then saturable binding requires ATP or a nonhydrolyzable analogue, and it correlates with DNA cleavage and inhibition of gyrase-mediated DNA supercoiling.

Quinolone binding to protein probably involves GyrA and ParC helix-4, which is located near the DNA gate region (Fig. 2). Amino acid substitutions causing the greatest decrease in susceptibility are located there (24, 36, 39, 43, 56, 84). Moreover, amino acid substitutions in helix-4 reduce quinolone binding (3), and replacing the helix-4-containing region of ParC with that of GyrA (61) or the *Escherichia coli* GyrA helix-4 with the comparable region from *Staphylococcus aureus* (74) causes quinolone sensitivity to more closely resemble that of the replacing region.

The orientation of quinolone binding to helix-4 can be inferred from interactions between particular amino acids and quinolone substituents. For example, a strain of *Mycobacterium smegmatis* containing a cysteine substitution at the N terminus of GyrA helix-4 is less susceptible to quinolones in which the C-7 ring contains an ethyl attached to the distal nitrogen (PD161144, Fig. 3) than to compounds in which the ethyl is attached to the side of the C-7 ring (PD161148, Fig. 3) (72). Bacterial strains with other amino acid substitutions in helix-4 fail to distinguish the two fluoroquinolones. These data indi-

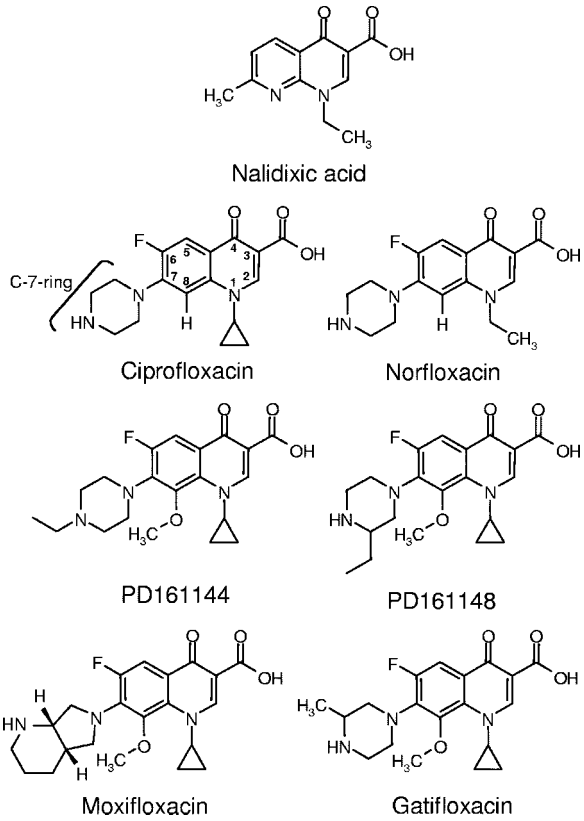

FIG. 3. Quinolone structures. Positions in the core ring structure are numbered for ciprofloxacin.

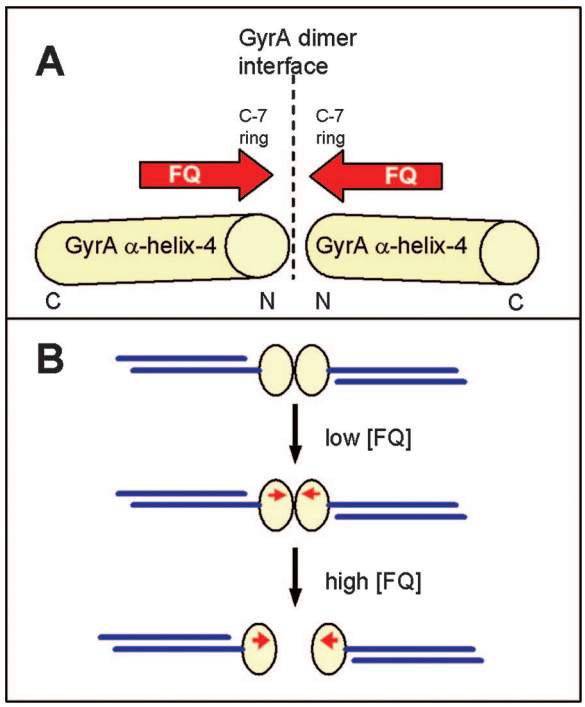

FIG. 4. Fluoroquinolone orientation and cleaved-complex destabilization. (A) Proposed orientation of fluoroquinolone on helix-4 of GyrA dimer. Fluoroquinolones are shown as arrows, with the arrowheads representing the C-7 ring moieties. Each cylinder represents the α-helix-4 of a GyrA subunit (N and C indicate the amino- and carboxy-terminal orientations of helix-4, respectively). The dashed line indicates the axis of symmetry at the GyrA dimer interface. Elements of the figure are not drawn to scale. (B) Proposed chromosome fragmentation arising from cleaved complex destabilization by some fluoroquinolones. Two GyrA subunits, shown as a dimer with GyrB omitted for clarity, are attached covalently to the 5′ end of cleaved DNA. Fluoroquinolone at a moderate concentration (arrow) binds to helix-4 and traps gyrase on DNA. At higher fluoroquinolone concentration, the GyrA subunits separate, thereby fragmenting the chromosome.

cate a negative interaction between an N-terminal cysteine of helix-4 and the distal ethyl group of PD161144. Such an interaction suggests that quinolones are oriented on the helix-4 with the C-7 ring pointing toward the N terminus of the helix, which would be toward the GyrA-GyrA (or ParC-ParC) dimer interface (Fig. 4A). While additional work is required to firmly establish this orientation, it explains why a Gly81-to-Asp mutation near the N terminus of helix-4 of *E. coli* GyrA confers resistance to ciprofloxacin but not to nalidixic acid (5), a quinolone that lacks a C-7 ring. The proposed orientation fits with ideas about lethal chromosome fragmentation, as discussed below.

GyrB undergoes a conformational change (34) during the DNA strand-passage reaction that may bring distal regions of the protein toward the DNA gate region of GyrA (discussed in reference 22). Two amino acid substitutions in GyrB, D426N and K447E, interfere with drug binding and quinolone-mediated inhibition of DNA supercoiling, relaxation, and DNA cleavage (22). These two amino acid changes, which are associated with resistance (82, 83), may be part of the drug-binding pocket. However, in the drug-free model of gyrase they are not located adjacent to the GyrA protein. It appears that D426 and K447 move to the DNA gate region during the GyrB conformational transition (15, 22).

In summary, the quinolones keep the DNA gate open in cleaved complexes, probably through binding to GyrA (ParC) helix-4, regions of GyrB (ParE), and DNA. Conformational energy associated with alterations in the structure of DNA and gyrase or topoisomerase IV may contribute to the complexes being reversible.

INHIBITION OF DNA REPLICATION

One of the consequences of cleaved complex formation is inhibition of nucleic acid biosynthesis. In the case of DNA replication, inhibition occurs within minutes of drug addition when gyrase is the target (20, 73). When topoisomerase IV is the target, inhibition of replication occurs slowly (39). We begin by discussing situations involving gyrase; at the end of the section we consider topoisomerase IV-mediated effects.

In cell-free systems containing gyrase, both quinolone binding and DNA cleavage are required to block DNA replication (78). The replication fork movement then halts 10 bp upstream from the DNA cleavage sites (78). Cleavage sites are also close to where replication fork blockage is seen in plasmid DNA extracted from norfloxacin-treated cells (64). Thus, gyrase-mediated inhibition of DNA synthesis appears to arise from collision of replication forks with cleaved complexes, which explains correlations between inhibition of DNA synthesis and cleaved complex formation in drug-treated cells (73).

Rapid inhibition of DNA synthesis is reversible (20); thus, it cannot be the immediate cause of cell death. Consistent with this conclusion, inhibition of DNA synthesis correlates with bacteriostatic drug susceptibility (i.e., the MIC) (8, 63), while it fails to correlate with rapid cell death in terms of kinetics, quinolone concentration, or interference through the inhibition of protein synthesis (7, 53). Moreover, situations have been found in which quinolones block DNA replication and cell growth with only some derivatives killing rapidly (52). Finally, the lethal action of nalidixic acid and ciprofloxacin does not depend on the compounds actively blocking DNA replication: both drugs kill *E. coli* even after replication is inhibited by shifting a temperature-sensitive *dnaB* mutant to high temperature (85).

Quinolone action contrasts with that of camptothecin, an inhibitor of eukaryotic topoisomerase I. For camptothecin, collision of replication forks with cleaved complexes generates lethal DNA breaks (31). Work with a cell-free system showed that this double-strand DNA breakage depends on the orientation of the complexes relative to the replication fork (76): in one orientation the topoisomerase-generated nick appears to be released from constraint by the replication fork, while in the other orientation it is not. In contrast, collision of replication forks and replication helicases with gyrase and topoisomerase IV trapped by quinolones fails to release DNA breaks (26, 67, 68, 78). The two bacterial enzymes appear to prevent replication forks from reaching the trapped DNA nicks from either side (discussed in reference 69). When a helicase forces its way through a quinolone-containing cleaved complex, as is seen with RuvAB, DNA nicks are reversed and not released from constraint (69). Thus, rapid quinolone-mediated cell death is probably not due simply to DNA breakage associated with collision of replication forks with cleaved complexes.

A context is still needed for the finding that replication-associated breaks occur in plasmid DNA after quinolone treatment. Pohlhaus and Kreuzer (64) developed an intracellular system in which plasmid replication is halted by high concentrations of norfloxacin (20-fold MIC). Within 6 min after drug addition, a distinct cleaved form of plasmid DNA is observed that is not reversed when cells are incubated under resealing conditions (EDTA plus incubation at 65°C). This cleaved DNA form is thought to arise from replication fork breakage, perhaps by an endonuclease acting on DNA extruded by a blocked replication fork (27, 64). Such cleavage could provide RecBC with access to circular DNA, as required for induction of the SOS response (6, 58). Association of replication-mediated cleavage with the lethal action of norfloxacin requires additional work that considers downstream events requiring ongoing RNA and protein synthesis (29, 53).

With topoisomerase IV, the rate at which replication is inhibited is 50 to 100 times slower than with gyrase (39). The difference is generally explained by topoisomerase IV functioning behind replication forks (38), while gyrase is probably ahead of them (13, 38). Creation of additional replication forks through deletion of *seqA* renders topoisomerase IV-mediated inhibition rapid, as if topoisomerase IV behind one fork is ahead of another that in the mutant started prematurely (38). Thus, it is likely that cleaved complexes containing topoisomerase IV are capable of blocking replication forks (38, 68), but inhibition is slow due to topoisomerase IV location. Nevertheless, topoisomerase IV can be the primary quinolone target. With gram-positive bacteria such as *S. aureus*, in vitro reactions with norfloxacin and gyrase fail to block replication fork movement (25). That explains why norfloxacin inhibits DNA replication largely through topoisomerase IV with *S. aureus* (16).

While most data argue against rapid cell death arising from quinolone-mediated inhibition of DNA replication, blocked replication forks could stimulate secondary events that kill cells slowly. Indeed, when drug treatment is long (overnight with *E. coli*), cell death can occur at quinolone concentrations that are only twice MIC (rapid killing generally requires concentrations 5 to 10 times the MIC). Slow killing, which is poorly understood mechanistically, is commonly expressed by the parameter called the minimal bactericidal concentration. In the following sections we mention two types of secondary damage that might contribute to quinolone-mediated cell death. We then consider chromosome fragmentation, which has been studied only with gyrase.

SOS RESPONSE AND CELL FILAMENTATION

A consequence of quinolone treatment is the induction of the SOS regulon, a set of more than 30 genes controlled by the *lexA* repressor (37). One of the genes induced is *sfiA* (*sulA*), which encodes an inhibitor of cell division that causes *E. coli* cells to form long, filamentous structures (12, 19). Filamentation has been thought to contribute to quinolone lethality, since filamentation is maximal at quinolone concentrations that give maximal killing (63) and since *sfiA* mutants that reduce filamentation are reported to the lower killing rate for several quinolones (62). However, *sfiA*-dependent filamentation is clearly reversible (49). Thus, filamentation is unlikely to be a direct cause of rapid quinolone-mediated lethality (12). Whether filamentation and other consequences of the SOS response contribute to slow death has not been studied.

The availability of *lexA* mutations that prevent induction of the SOS response allows the contribution of the response to be examined with various quinolone derivatives. For the potent fluoroquinolones, rapid lethality is increased by a *lexA* Ind$^-$ allele, while under the same conditions the *lexA* mutation has no effect on nalidixic acid-dependent killing (30, 45). Moreover, nalidixic acid lethality shifts from being unaffected by the *lexA* Ind$^-$ mutation in a *gyrA*$^+$ background to being 10-fold enhanced in a *gyrA* mutant thought to have an altered GyrA-GyrA interface (53). Thus, the quinolones appear to produce at least two types of lethal damage that can be distinguished by protective elements of the SOS response.

TOXIN-ANTITOXIN MODULES AND PROGRAMMED CELL DEATH

Toxin-antitoxin modules may also contribute to quinolone lethality. These modules appear to be part of the bacterial stress response, acting when elimination of a short-lived antitoxin allows the cognate toxin to interfere with the bacterial transcription-translation machinery (14, 18). It has been suggested that activation of some toxins leads to programmed cell death, an irreversible series of events that causes the loss of colony-forming ability even after removal of the stressor (14, 21, 65). Several types of stress result in toxin-mediated cell

death. One is nalidixic acid treatment at 500 to 1,000 times the MIC for 10 min. A modest (90%) decrease in the number of CFU follows unless the *chpAIK* (*mazEF*) toxin-antitoxin module is absent (40). In wild-type cells, high-level expression of the antitoxin *chp*AI (*mazE*) reverses lethality if applied within 90 min after drug treatment, but antitoxin is ineffective if its expression is induced later. Thus, a critical time window for reversal exists. A similar behavior is observed with a variety of stresses, suggesting that the response is general. Although the 10-min treatment with nalidixic acid is probably sufficient to form cleaved complexes and to block DNA replication (73), it has not been established that the very high concentrations of nalidixic acid (21, 40) cause lethal effects mediated only by the topoisomerases. Additional information is needed to relate toxin-mediated cell death to other lethal effects of quinolones.

Generation of hydroxyl radicals, high levels of ATP, and depletion of NADH pools may also contribute to programmed cell death after norfloxacin treatment. Defects in several genes involved in pathways leading to these events (*acnB*, *atpC*, *fur*, *icdA*, and *iscS*) lower the lethal activity of norfloxacin, and deletion of *sodB* raises it. Since the *sodB* effect is slow while norfloxacin kills *E. coli* rapidly, production of oxidative species is likely to be a secondary effect associated with a variety of lethal antimicrobials. It is not known whether these events occur when norfloxacin lethality is blocked by inhibitors of protein synthesis or when quinolones kill anaerobically (see below).

CHROMOSOME FRAGMENTATION

Since cleaved complexes are distributed throughout the chromosome, the release of DNA breaks from constraint is expected to fragment the chromosome into small pieces. Initial support for this hypothesis came from a chromosome supercoiling study. When *E. coli* is treated with concentrations of oxolinic acid sufficient to form cleaved complexes and block DNA replication, DNA supercoils are observed in isolated nucleoids (73). However, if quinolone concentrations are raised to levels that kill cells rapidly, supercoils are absent from nucleoids, even in the presence of an intercalating dye that would otherwise introduce supercoils (7). Since nucleoid supercoiling is partitioned into many topologically independent domains (11, 81), the inability to maintain supercoiling is most easily explained by widespread chromosome fragmentation (release of DNA breaks from constraint). When inhibition of protein synthesis blocks quinolone-mediated lethality, it also blocks quinolone-mediated loss of supercoiling (7). Thus, chromosome fragmentation correlates with cell death.

More direct assays of chromosome fragmentation now support the supercoiling experiments. One involves measuring cell lysate viscosity after the treatment of bacteria with quinolone. With *E. coli*, gently prepared cell lysates exhibit little viscosity; subsequent heating unfolds the nucleoids and causes lysates to become very viscous. If chromosomes are fragmented by quinolone action, lysates would fail to become viscous when heated (53). Another assay involves sedimentation of DNA into sucrose density gradients: broken DNA sediments very slowly. To minimize DNA aggregation, sucrose gradients contain SDS, which would artificially release constrained breaks (Fig. 1, step g); consequently, sedimentation experiments generally involve preincubation of lysates under resealing conditions (addition of EDTA) to identify irreversible breaks.

Both viscosity and sedimentation measurements reveal that nalidixic acid causes irreversible chromosome fragmentation with kinetics similar to cell death and much slower than inhibition of DNA replication (53). Additional correlations are revealed by two environmental perturbations: anaerobic growth and inhibition of protein synthesis by chloramphenicol. For first-generation quinolones, both perturbations block lethal action and chromosome fragmentation (51, 53). However, neither prevents cleaved complex formation, as shown by the presence of DNA breakage when cell lysates are treated with SDS. Thus, quinolone lethality correlates with chromosome fragmentation, while DNA breaks can be found in cleaved complexes even when cells are not killed.

The inhibitory effect of chloramphenicol on killing by nalidixic acid and norfloxacin suggests that a suicide factor is involved (Fig. 1, steps d and e). Such a factor is likely to be short-lived, since the lethal action of nalidixic acid is rapidly blocked by chloramphenicol or by a shift to anaerobiosis (10, 51). Although mutants have been obtained that block nalidixic acid lethality without affecting bacteriostatic action (80; X. Zhao, unpublished observation), the genes involved have proven difficult to identify. Suicide factors are unlikely to be part of the SOS response because a *lexA* Ind^- mutation can have no effect on lethality caused by nalidixic and oxolinic acids (45, 53).

The lethal activity of norfloxacin is also blocked by chloramphenicol (29). However, unlike nalidixic acid, norfloxacin kills cells after anaerobic shock (51) or after suspension of cells in cold saline (29). Moreover, lethal action is increased if the SOS response is blocked (30). Thus, norfloxacin action (Fig. 1, step e) is distinct from that of nalidixic acid (Fig. 1, step d). The molecular basis of this difference has not been determined.

DESTABILIZATION OF CLEAVED COMPLEXES

The chromosome fragmentation described above, which depends on a suicide factor, cannot be the only way that quinolones kill cells rapidly because many fluoroquinolones are lethal even in the absence of protein synthesis (7, 46, 47, 53). As pointed out above, the lethal activity of potent fluoroquinolones is increased by a *lexA* Ind^- allele, unlike the activity of first-generation quinolones (30, 45). Thus, at least two modes of rapid killing must exist: one that requires ongoing protein synthesis (Fig. 1, steps d and e) and one that does not (Fig. 1, step f). It has been suggested that step f involves destabilization of cleaved complexes, since that would not require ongoing protein synthesis (7, 53). A prediction of the destabilization hypothesis is that chromosome fragmentation will be observed in cell-free systems. Such is the case when isolated nucleoids are incubated with purified gyrase plus gatifloxacin (53).

A potential outcome of cleaved complex destabilization is dissociation of GyrA subunits attached to the ends of DNA (Fig. 4B). Such an idea emerged from studies of quinolone-stimulated illegitimate recombination (32, 33, 66), a phenomenon that is best explained by gyrase subunit dissociation-reassociation (this concept also appears to apply to interactions of eukaryotic topoisomerase II with certain antitumor agents [2]). If quinolones stimulate dissociation of GyrA

subunits, an amino acid substitution located on the GyrA-GyrA dimer interface might promote it. This prediction was tested by examining an *E. coli* mutant in which Ala-67 (Fig. 2) is replaced with Ser. This amino acid substitution, which confers a modest fourfold increase in MIC, allows nalidixic acid to kill *E. coli* and fragment chromosomes in the presence of chloramphenicol (53). With wild-type cells both events are blocked by chloramphenicol. Moreover, when isolated nucleoids are incubated with mutant gyrase, nalidixic acid causes chromosome fragmentation, which is not seen with wild-type gyrase and nalidixic acid (53). Finally, a noninducible *lexA* mutation renders *E. coli* hypersusceptible to nalidixic acid in the *gyrA* A67S mutant, a phenomenon not observed with *gyrA*$^+$ cells (53). Thus, the mutation appears to shift lethal activity from step d in Fig. 1 to step f, perhaps by the alanine-to-serine change weakening hydrophobic interactions between the GyrA subunits.

The connection between cleaved complex destabilization and chloramphenicol-insensitive killing (Fig. 1, step f) allows conclusions to be drawn about effects of quinolone structure. For example, the N-1 cyclopropyl group is probably important for cleaved complex destabilization, since this moiety is the only difference between ciprofloxacin, a compound that kills in the presence of chloramphenicol, and norfloxacin, a compound that does not (29, 51). A C-8-methoxy group can also be important, since it increases chloramphenicol-insensitive killing of fluoroquinolones with *E. coli* (87), *S. aureus* (86), *M. smegmatis* (52), and *M. tuberculosis* (50). A third factor is the structure of the C-7 substituent. With mycobacteria, the large C-7 ring system of moxifloxacin (Fig. 3) increases chloramphenicol-insensitive lethality relative to that of another C-8-methoxy compound, gatifloxacin (50). With *E. coli*, the C-7 N-ethyl piperazine of PD161144 (Fig. 3) confers greater chloramphenicol-insensitive lethal activity than a C-7 C-ethyl derivative (PD161148 [53]). We speculate that the quinolone C-7 rings point toward each other across a GyrA-GyrA interface (Fig. 4A) and that particular C-7 rings have a negative interaction. Such an interaction might destabilize cleaved complexes and ultimately lead to gyrase subunit dissociation (Fig. 4B).

CONCLUDING REMARKS

It now appears that inhibition of replication is an unlikely source of rapid killing by the quinolones, since inhibition of replication is neither necessary nor sufficient for quinolone-mediated rapid cell death and since quinolone-mediated replication inhibition and cell death fail to correlate with respect to kinetics, quinolone concentration, and the effects of protein synthesis inhibitors. Nor is blockage of RNA polymerase movement by cleaved complexes (79) likely to be lethal, since blockage does not fragment DNA (79) and since inhibition of RNA synthesis is protective (46). We propose chromosome fragmentation as an alternative source of rapid death. According to this hypothesis, quinolone lethality can be described as a two-step process in which the first step is reversible (bacteriostatic) formation of cleaved complexes. This step blocks bacterial DNA replication, induces the SOS response, and leads to cell filamentation (Fig. 1, pathway c). Although these events do not appear to be rapidly lethal, their involvement in slow death has not been ruled out. In a second, lethal step that requires higher quinolone concentrations, DNA breaks are released from constraint by at least two processes. One (Fig. 1, steps d and e) requires protein synthesis; the other (Fig. 1, step f) does not. The relative contribution of each pathway to cell death depends on quinolone structure, with the older quinolones requiring protein synthesis and some new fluoroquinolones functioning without it. This dichotomy between steps d and f extends to fragmentation of nucleoids in vitro and to protective effects of the SOS response. Norfloxacin (step e) appears to represent an intermediate situation in which some properties are similar to step d and others are similar to step f. How norfloxacin-mediated replication fork breakage (64) fits into these categories is not currently understood.

The chromosome fragmentation hypothesis requires much more testing: proteins involved in steps d and e of Fig. 1 need to be identified, the cell-free test for step f needs to be extended to plasmid systems that can be readily studied, and the effects of high drug concentration, including the paradoxical loss of lethality at very high concentrations (10), need to be explained in molecular terms. Nevertheless, the scheme in Fig. 1 has implications for developing new quinolones. For example, the distinction between blocking growth (Fig. 1, step b) and cell death (Fig. 1, steps c, d, and e) means that a compound with a low MIC is not necessarily highly lethal. Indeed, a ciprofloxacin dimer blocks growth of mycobacteria while failing to kill, even at a high concentration (52). The distinction also means that quinolones can be compared for lethal activity independent of drug uptake, efflux, and the ability to form cleaved complexes: by normalizing lethality to MIC, substituents that preferentially increase killing can be identified. Finally, quinolones that kill via pathway f may be lethal with nongrowing bacterial cells. Such a feature could be particularly important with pathogens, such as *M. tuberculosis*, that enter a dormant state in which they become tolerant to many antimicrobials (77).

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