Action of Quinolones against *Staphylococcus aureus* Topoisomerase IV: Basis for DNA Cleavage Enhancement†

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ABSTRACT: Topoisomerase IV is the primary cellular target for most quinolones in Gram-positive bacteria; however, its interaction with these agents is poorly understood. Therefore, the effects of four clinically relevant antibacterial quinolones (ciprofloxacin, and three new generation quinolones: trovafloxacin, levofloxacin, and sparfloxacin) on the DNA cleavage/religation reaction of *Staphylococcus aureus* topoisomerase IV were characterized. These quinolones stimulated enzyme-mediated DNA scission to a similar extent, but their potencies varied significantly. Drug order in the absence of ATP was trovafloxacin > ciprofloxacin > levofloxacin > sparfloxacin. Potency was enhanced by ATP, but to a different extent for each drug. Under all conditions examined, trovafloxacin was the most potent quinolone and sparfloxacin was the least. The enhanced potency of trovafloxacin correlated with several properties. Trovafloxacin induced topoisomerase IV-mediated DNA scission more rapidly than other quinolones and generated more cleavage at some sites. The most striking correlation, however, was between quinolone potency and inhibition of enzyme-mediated DNA religation: the greater the potency, the stronger the inhibition. Dose−response experiments with two topoisomerase IV mutants that confer clinical resistance to quinolones ($GrlA^{Ser80Phe}$ and $GrlA^{Glu84Lys}$) indicate that resistance is caused by a decrease in both drug affinity and efficacy. Trovafloxacin is more active against these enzymes than ciprofloxacin because it partially overcomes the effect on affinity. Finally, comparative studies on DNA cleavage and decatenation suggest that the antibacterial properties of trovafloxacin result from increased *S. aureus* topoisomerase IV-mediated DNA cleavage rather than inhibition of enzyme catalysis.

Eubacteria encode two distinct type II topoisomerases, DNA gyrase and topoisomerase IV (*1*−*3*). DNA gyrase is the only known topoisomerase that is capable of actively underwinding covalently closed DNA molecules (*4*). This enzyme plays critical roles in DNA replication, recombination, and transcription, as well as the maintenance of genomic superhelical density (*2*, *5*−*9*). Topoisomerase IV is a highly active decatenase and is required for the segregation of daughter chromosomes (*3*, *10*−*16*). Due to their irreplaceable physiological functions, both enzymes are essential to cell survival (*1*−*3*).

Beyond their cellular duties, DNA gyrase and topoisomerase IV are also the targets for quinolone-based antibacterials (*3*, *17*−*30*). Quinolones are in wide use for the treatment of infections in humans and display the greatest activity and range of any oral antibacterials currently in clinical use (*31*−*34*). These drugs do not kill bacterial cells by robbing them of the essential functions of DNA gyrase or topoisomerase IV (at least in Gram-negative species). Rather, they act by increasing levels of covalent enzyme-cleaved DNA complexes that are normal intermediates in the catalytic cycles of both type II enzymes (*3*, *5*, *8*, *20*, *29*, *33*−*37*). Consequently, they convert DNA gyrase and topoisomerase IV into potent cellular toxins that generate double-stranded breaks in the chromosomes of treated bacteria (*3*, *8*, *34*, *35*, *37*).

DNA gyrase and topoisomerase IV from Gram-negative and -positive species display differential sensitivity to quinolones. Early quinolones were developed primarily for high activity against DNA gyrase (*3*, *17*, *18*, *30*, *33*, *34*). Since Gram-positive DNA gyrase displays a natural resistance to members of this drug family, quinolones such as ciprofloxacin have been used predominately to treat Gram-negative infections (*3*, *33*, *34*, *38*). However, the development of "new generation" quinolones that display increased activity toward Gram-positive topoisomerase IV has significantly broadened the clinical spectrum of this drug family (*3*, *33*, *34*, *39*−*41*). As a result, family members such as trovafloxacin are now used to treat many Gram-positive respiratory infections that previously were resistant to quinolone antibacterials (*3*, *33*, *34*, *42*−*44*).

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Despite the emergence of Gram-positive topoisomerase IV as an important pharmaceutical target, its interactions with quinolones have not been well-defined. Therefore, the effects of four clinically important quinolones on the DNA cleavage/religation reaction of *Staphylococcus aureus* topoisomerase IV were characterized. Results indicate a strong correlation between the potency of quinolone-induced DNA scission and the ability to inhibit religation of cleaved nucleic acid molecules. These findings support the hypothesis (*45*) that quinolones act primarily by blocking the DNA religation reaction of Gram-positive topoisomerase IV.

EXPERIMENTAL PROCEDURES

Topoisomerase IV was cloned from *S. aureus* 4220, overexpressed as the separate subunits (GrlA and GrlB) in *Escherichia coli*, and purified by a modification (*45*) of the procedure of Hallett et al. (*46*).

Ciprofloxacin was purchased from Sigma. All other quinolones were obtained from Pfizer Central Research. Ciprofloxacin and sparfloxacin were stored as 40 and 20 mM stock solutions, respectively, in 0.1 N NaOH at −20 °C, then diluted one-fifth with 10 mM Tris-HCl, pH 7.9, immediately prior to use. Trovafloxacin and levofloxacin were stored as 20 mM stock solutions in water at −20 °C. All other chemicals were of analytical reagent grade.

Preparation of DNA Substrates. Negatively supercoiled pBR322 DNA was isolated from *E. coli* as described previously (*47*). Kinetoplast DNA (kDNA)[1] was isolated from *Crithidia fasiculata* as described (*48*). A 564-bp DNA substrate (residues 376−939 in pBR322), uniquely end-labeled with ^{32}P, was prepared as described by Burden et al. (*49*).

DNA Cleavage. DNA cleavage assays were performed as described by Corbett et al. (*50*). Briefly, 5 nM negatively supercoiled pBR322 DNA was incubated in the absence or presence of 1 mM ATP with 15 nM topoisomerase IV and 0−10 μM quinolone in 20 μL of cleavage buffer (35 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 5 mM DTT, and 50 μg/mL bovine serum albumin) at 37 °C. Unless stated otherwise, reactions were carried out for 30 min. DNA cleavage reactions were stopped by the addition of SDS (0.5% final concentration) followed by EDTA (15 mM final concentration). Samples were digested with proteinase K (80 μg/mL final concentration) for 30 min at 45 °C. Following the addition of 60% sucrose, 0.5% bromophenol blue, and 0.5% xylene cyanole FF in 10 mM Tris-HCl, pH 7.9, DNA products were resolved by electrophoresis in 1% agarose gels in 40 mM Tris-acetate, pH 8.3, 2 mM EDTA, and 0.5 μg/mL ethidium bromide. DNA bands were visualized by UV light, photographed through Kodak 23A and 12 filters with Polaroid type 665 film, and quantitated by scanning negatives with an E−C apparatus model EC910 densitometer in conjunction with Hoefer GS-370 Data System software. The intensity of bands in the negative was proportional to the amount of DNA present. Double-stranded DNA breaks were monitored by the conversion of negatively supercoiled plasmid to linear molecules.

Sites of DNA cleavage were determined in the absence or presence of drugs using the protocol of Burden et al. (*49*). Assay mixtures contained 1.4 nM labeled linear 564-mer DNA (25 ng), 6 nM topoisomerase IV, and 0 or 10 μM quinolone in cleavage buffer that contained 1 mM ATP and were incubated at 37 °C for 15 min. DNA cleavage complexes were trapped by the addition of SDS, and samples were digested with proteinase K in the presence of EDTA, as described above. DNA cleavage products were precipitated twice with ethanol, dried, and resuspended in 40% formamide, 8.4 mM EDTA, 0.02% bromophenol blue, and 0.02% xylene cyanole FF. Samples were subjected to electrophoresis in 8% sequencing gels (*51*), fixed in 10% methanol/10% acetic acid, and dried. Reaction products were visualized using a Molecular Dynamics PhosphorImager.

DNA Religation. Reactions were carried out by the procedure of Anderson et al. (*36*). This assay is based on the finding that topoisomerase IV can religate, but not cleave DNA at suboptimal temperatures (*36*). Topoisomerase IV-mediated DNA cleavage/religation equilibria with negatively supercoiled pBR322 were established in the presence of ATP and 0 or 5 μM quinolone as described in the preceding section. Religation was initiated by shifting the temperature from 37 to 65 °C and stopped at various times up to 120 s by the addition of SDS (0.5% final concentration). Samples were processed and analyzed by agarose gel electrophoresis as described in the preceding section. The apparent first-order rate of DNA religation was determined by quantifying the loss of linear molecules.

Decatenation Reactions. Assays were carried out by the procedure of Anderson et al. (*36*). Briefly, reaction mixtures contained 5 nM kDNA, 7.5 nM topoisomerase IV, 1 mM ATP, and 0−100 μM quinolone and were incubated at 37 °C for 15 min in 35 mM Tris-HCl, pH. 7.9, 10 mM $MgCl_2$, 350 mM potassium glutamate, 5 mM DTT, and 50 μg/mL bovine serum albumin. Reactions were terminated by the addition of 3 μL of 0.77% SDS/77 mM EDTA and digested with proteinase K as above. DNA products were resolved by electrophoresis in 1% agarose gels in 100 mM Tris-borate, pH 8.3, 2 mM EDTA, stained with 1 μg/mL ethidium bromide, and visualized as described above. The percent of decatenation was quantified by determining the appearance of monomeric circular DNA minicircles.

RESULTS

Although topoisomerase IV is the primary cellular target for most quinolones in Gram-positive bacteria (*3*, *21*, *24*−*26*, *30*, *33*, *34*, *52*), its interaction with these agents is poorly understood. Therefore, the effects of four clinically relevant quinolone antibacterials on the DNA cleavage/religation equilibrium of *S. aureus* topoisomerase IV were characterized.

The quinolones chosen for this study were ciprofloxacin, as well as three new generation quinolones, trovafloxacin, levofloxacin, and sparfloxacin (Figure 1). Ciprofloxacin is considered to be the most active and broad spectrum oral antibacterial used against Gram-negative infections (*31*−*34*). The new generation quinolones were developed for their extended range against Gram-positive bacteria (*3*, *33*, *34*). Of these, trovafloxacin displays the greatest activity against human respiratory pathogens (*39*, *43*, *52*). In addition,

[1] Abbreviations: kDNA, kinetoplast DNA; CC_2 value, concentration of drug required to double levels of DNA cleavage mediated by topoisomerase IV; IC_{50} value, concentration of drug required to inhibit topoisomerase IV-catalyzed DNA decatenation by 50%.

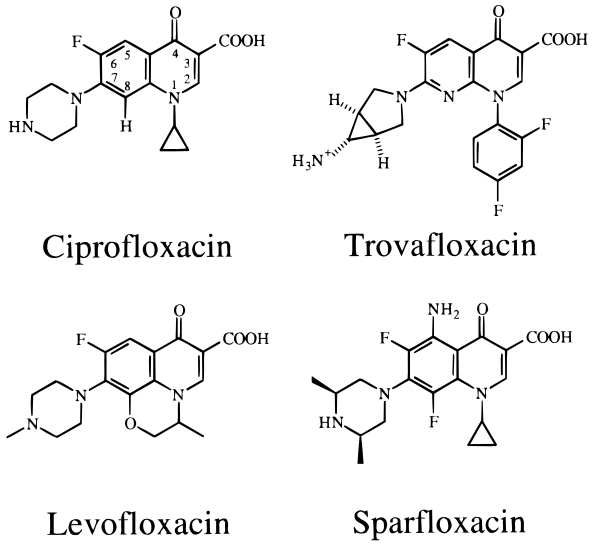

FIGURE 1: Quinolone structures. The ring numbering system shown for ciprofloxacin is applicable to all of the quinolones.

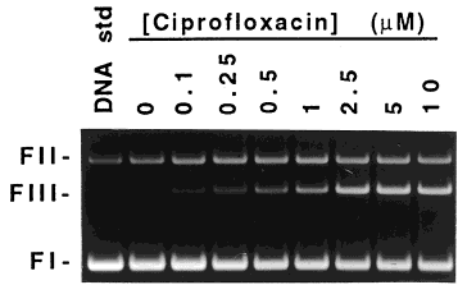

FIGURE 2: Stimulation of topoisomerase IV-mediated DNA cleavage by ciprofloxacin. An ethidium bromide-stained agarose gel is shown. Double-stranded DNA cleavage converts negatively supercoiled plasmid (form I, FI) to linear molecules (form III, FIII). The position of nicked circular DNA (form II, FII) is shown for reference.

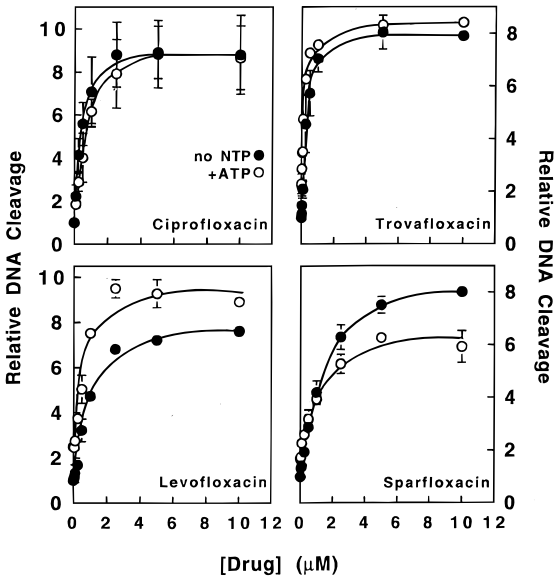

FIGURE 3: Quinolones enhance *S. aureus* topoisomerase IV-mediated DNA scission. Assays containing 15 nM topoisomerase IV, 5 nM pBR322 plasmid DNA, and 0 to 10 μM drug were incubated in the absence (●) or presence (○) of 1 mM ATP. The relative level of DNA cleavage in the absence of drug was set to 1.0. Data represent the average of three independent experiments. Standard deviations are shown as error bars.

Table 1: Effects of ATP on Quinolone Potency

drug	CC_2[a] (nM) −ATP	CC_2[a] (nM) +ATP
trovafloxacin	90	10
levofloxacin	250	40
ciprofloxacin	150	70
sparfloxacin	270	110

[a] The concentration of quinolone required to double levels of DNA cleavage.

sparfloxacin is the only quinolone that appears to preferentially target DNA gyrase over topoisomerase IV in Gram-positive species (*39*, *40*, *53*).

Stimulation of Topoisomerase IV-Mediated DNA Cleavage by Quinolones. All four quinolones examined stimulated DNA cleavage mediated by *S. aureus* topoisomerase IV ~6−8-fold. A representative agarose gel for ciprofloxacin is shown in Figure 2; quantitated results for all of the drugs are shown in Figure 3. Maximal cleavage levels (i.e., drug efficacy) were similar in the presence or absence of ATP.

In contrast, quinolone potency varied significantly. The order observed in the absence of ATP was trovafloxacin > ciprofloxacin > levofloxacin > sparfloxacin (Figure 3 and Table 1). Potency was enhanced by ATP, but to a different extent for each drug (Figure 3 and Table 1). While the activities of trovafloxacin and levofloxacin rose nearly an order of magnitude in the presence of the nucleotide triphosphate, those of ciprofloxacin and sparfloxacin increased only 2-fold. Consequently, in the presence of ATP, trovafloxacin was still the most potent quinolone against *S. aureus* topoisomerase IV and sparfloxacin, the least. Due to the differential effects of ATP on quinolone action, the potency of levofloxacin was higher than that of ciprofloxacin in the presence of the high-energy cofactor.

Basis for DNA Cleavage Enhancement by Quinolones. The mechanistic basis for potency differences among quinolones is not known. Therefore, three independent experiments were carried out to address this important issue.

In the first, preequilibrium rates of topoisomerase IV-mediated DNA scission were compared for trovafloxacin and ciprofloxacin. Since quinolone potency most likely reflects the kinetic affinity of these drugs for the enzyme·DNA complex, it is possible that differences in potency reflect differences in the rate of DNA cleavage. As seen in Figure 4, rates of DNA scission in the presence of trovafloxacin were ~2 times higher than those observed for ciprofloxacin. Although the magnitude of this difference is smaller than that observed for potency (~7-fold), it suggests a relationship between the kinetic affinity of quinolones and the rate at which they are able to form a ternary topoisomerase IV·drug·DNA cleavage complex.

In the second experiment, sites of DNA cleavage were mapped for the four quinolones to determine whether differences in quinolone action in the presence of ATP correlate with site specificity. As seen in Figure 5, similar DNA cleavage maps were generated for all of the quinolones examined, but site utilization differed significantly. Trovafloxacin, which was the most potent quinolone, generated considerably more cleavage at some sites. Furthermore, sparfloxacin, which was the least potent member of the series, induced very little (or no) cleavage at many sites that were prominent for the other quinolones. Finally, cleavage site

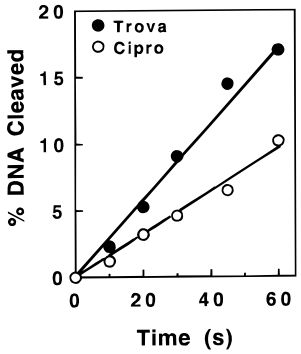

FIGURE 4: Time course for quinolone-induced DNA cleavage mediated by topoisomerase IV. Assays contained 5 μM trovafloxacin [Trova (●)] or ciprofloxacin [Cipro (○)]. ATP was included in assays because of the larger difference in potency between the two quinolones in the presence of the nucleotide triphosphate. Data are presented as the percent of the initial supercoiled DNA substrate cleaved and represent the average of two independent experiments. Standard errors varied less than 0.3%.

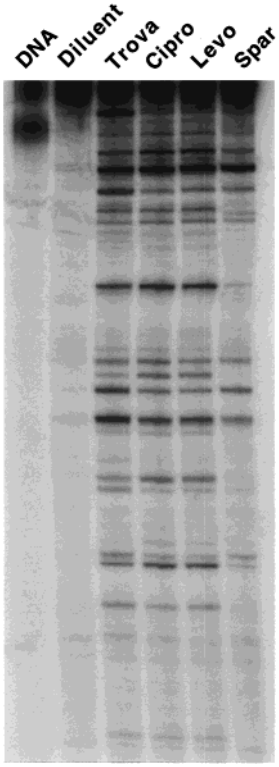

FIGURE 5: Effects of quinolones on site-specific DNA cleavage mediated by topoisomerase IV. Assays containing 6 nM topoisomerase IV and 1.4 nM labeled linear 564-mer substrate (DNA) were incubated in the absence (Diluent) or presence of 10 μM quinolone (trovafloxacin, Trova; ciprofloxacin, Cipro; levofloxacin, Levo; sparfloxacin, Spar).

utilization for ciprofloxacin and levofloxacin, which displayed similar potencies, was comparable and was intermediate to that of the other two quinolones. Thus, quinolone potency may be related to the distribution of drug-induced DNA sites cleaved by topoisomerase IV.

A recent study on the effects of ciprofloxacin on *S. aureus* topoisomerase IV suggested that quinolones enhanced enzyme-mediated DNA scission primarily by inhibiting the religation of cleaved nucleic acids (*45*). Since the quinolones utilized in the present work differ in potency by an order of magnitude, they present a novel opportunity to test this hypothesis. Therefore, in the third experiment, rates of DNA

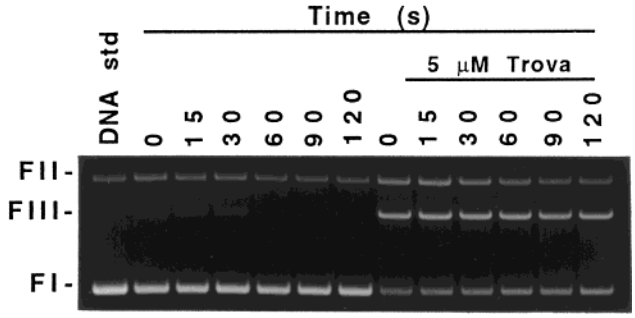

FIGURE 6: Inhibiton of topoisomerase IV-mediated DNA religation by trovafloxacin. An ethidium bromide-stained agarose gel is shown for assays carried out in the absence or presence of 5 μM trovafloxacin (Trova). DNA religation was monitored by the loss of linear molecules (form III, FIII). The positions of negatively supercoiled plasmid (form I, *FI*) and nicked circular DNA (form II, FII) are shown for reference.

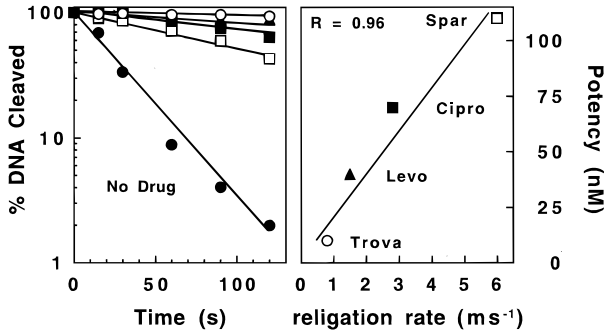

FIGURE 7: Quinolones inhibit DNA religation mediated by topoisomerase IV. (Left panel) Assays were carried out the absence of drug (●) or in the presence of 5 μM trovafloxacin (○), levofloxacin (▲), ciprofloxacin (■), or sparfloxacin (□). Religation was initiated by shifting the temperature from 37 to 65 °C. At time 0, levels of double-stranded cleavage were set to 100%. Data represent the average of two independent experiments. Standard errors varied less than 0.2%. (Right panel) Drug potency (expressed as CC_2 values, see Table 1) was plotted vs religation rate.

religation were compared for ciprofloxacin, trovafloxacin, levofloxacin, and sparfloxacin. A representative agarose gel for trovafloxacin is shown in Figure 6; quantitated results for all of the drugs are shown in Figure 7. Consistent with the previous study, all of the quinolones significantly decreased rates of topoisomerase IV-mediated DNA religation (Figure 7, left panel). Furthermore, there was a strong correlation ($R = 0.96$) between quinolone potency and inhibition of DNA religation (Figure 7, right panel). Thus, the slowest rate of religation was observed in the presence of the most potent quinolone, trovafloxacin, followed by levofloxacin, ciprofloxacin, and sparfloxacin, in that order. These results provide strong evidence that quinolones act on *S. aureus* topoisomerase IV primarily by inhibiting DNA religation and that the potency of drug action reflects the degree of this inhibition.

Basis for Quinolone Resistance. The development of quinolone-resistant bacteria is commonly associated with the generation of specific point mutations in either DNA gyrase or topoisomerase IV (*3*, *28*−*30*, *33*, *34*). The common feature of these mutations is their ability to impair quinolone stimulation of enzyme-mediated DNA cleavage (*3*, *28*, *29*). In clinical isolates of Gram-negative species, the most frequent resistance-conferring mutations are found in the A subunit of DNA gyrase (i.e., GyrA) (*3*, *28*−*30*, *33*, *34*).

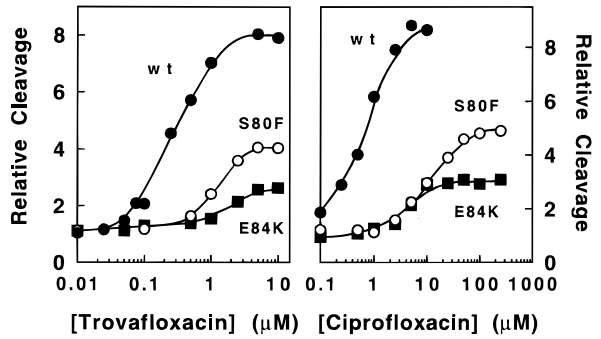

FIGURE 8: Effects of quinolones on topoisomerase IV mutant enzymes. Assays contained 5 nM pBR322 plasmid DNA and 15 nM of wild-type [wt (●)], Ser80 → Phe [S80F (○)], or Glu84 → Lys [E84K (■)] topoisomerase IV enzymes. Reactions also contained either 0−10 μM trovafloxacin (left panel) or 0−250 μM ciprofloxacin (right panel). The relative level of DNA cleavage in the absence of drug was set to 1.0. Data represent the average of three independent experiments. Standard deviations varied less than ∼0.2.

Table 2: Quinolone Resistance of Mutant Topoisomerase IV Enzymes

	relative potency[a]			relative efficacy[b]		
drug	wt	S80F	E84K	wt	S80F	E84K
ciprofloxacin	1.0 (0.5 μM)	0.05 (10 μM)	0.07 (7.0 μM)	1.0 (7.8)	0.5 (4.0)	0.3 (2.0)
trovafloxacin	1.0 (0.3 μM)	0.30 (1.0 μM)	0.27 (1.1 μM)	1.0 (7.0)	0.5 (3.2)	0.2 (1.6)

[a] Potency values are relative to those of the wild-type enzyme (set to 1.0). Numbers in parentheses represent the drug concentration required to reach 50% of the maximal level of DNA cleavage. [b] Efficacy values are relative to those of the wild-type enzyme. Numbers in parentheses represent the maximal level of DNA cleavage enhancement at saturating quinolone concentrations.

Conversely, in Gram-positive species, these mutations are most often found in the equivalent A subunit of topoisomerase IV (i.e., GrlA) (*3*, *29*, *30*, *33*, *34*). Despite the clinical impact of drug resistance, the decreased ability of quinolones to enhance DNA cleavage by these mutant enzymes is not well understood.

The only previous study that addressed this mechanistic issue demonstrated that the conversion of Ser83 to Trp in *E. coli* GyrA impaired the ability of the enzyme to bind norfloxacin (*54*). The present work employed dose−response analysis to assess the basis for the quinolone resistance of two mutant forms of *S. aureus* topoisomerase IV, Ser80 → Phe and Glu84 → Lys in GrlA, respectively. [Ser80 of GrlA is homologous to Ser83 in *E. coli* GyrA (*55*).] These alterations represent two of the most frequent resistance-conferring mutations found in clinical isolates of *S. aureus* (*33*, *39*, *56*).

As seen in Figure 8 and Table 2, both mutations impaired the ability of trovafloxacin (left panel) and ciprofloxacin (right panel) to stimulate topoisomerase IV-mediated DNA cleavage. The potency of trovafloxacin for each enzyme (as determined from the concentration of quinolone required to reach 50% saturation) was reduced ∼3-fold. The decrease in ciprofloxacin potency was considerably more severe, dropping more than an order of magnitude. These findings indicate that the Ser80 → Phe and Glu84 → Lys mutations decrease the affinity of topoisomerase IV for quinolones.

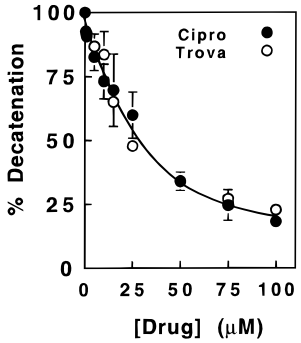

FIGURE 9: Inhibition of topoisomerase IV-catalyzed DNA decatenation by quinolones. Topoisomerase IV (7.5 nM) was incubated with 5 nM kDNA, 1 mM ATP, and 0 to 100 μM of either ciprofloxacin [Cipro (●)] or trovafloxacin [Trova (○)]. Data represent the average of two independent experiments. Standard errors are shown.

The efficacy of trovafloxacin and ciprofloxacin (as determined from the maximal level of cleavage induced by saturating drug concentrations) also was reduced for each mutant enzyme. In contrast to changes in potency, decreases in efficacy were similar for both quinolones. The Glu84 → Lys mutation caused the greater effect (∼3−5-fold), but a reduced efficacy also was observed for the Ser80 → Phe mutation (∼2-fold). These findings indicate that even after quinolones saturate the ternary topoisomerase IV·drug·DNA complex, their ability to increase nucleic acid cleavage is impaired by the mutations.

Effects of Quinolones on Topoisomerase IV Catalysis. Beyond their effects on DNA cleavage, quinolones inhibit the overall catalytic activity of type II topoisomerases (*19*, *20*, *22*, *36*, *57*−*59*). Previous studies with antibacterial and antineoplastic quinolones strongly suggest that these two effects are independent of one another and that inhibition of catalytic activity requires considerably higher drug concentrations (*36*, *37*, *57*, *58*).

To examine these relationships for the Gram-positive system, the effects of ciprofloxacin and trovafloxacin on the DNA decatenation activity of *S. aureus* topoisomerase IV were established. As seen in Figure 9, both quinolones inhibited catalytic activity to a similar extent, and IC_{50} values (∼30 μM) for each were approximately 2 orders of magnitude higher than the drug concentration required to increase DNA cleavage to 50% saturation levels (Table 2). These results are consistent with the previous studies on Gram-negative topoisomerase IV.

DISCUSSION

Topoisomerase IV from Gram-positive species appears to be the primary cytotoxic target for many quinolone-based drugs (*3*, *21*, *24*−*26*, *30*, *33*, *34*, *52*). Consequently, this enzyme is rapidly emerging as an important focal point for antibacterial drug discovery. Before the clinical potential of new generation quinolones can be fully realized, however, it is necessary to define the mechanistic basis for their actions against Gram-positive topoisomerase IV. Therefore, the present study characterized interactions between four clinically relevant quinolones and this type II enzyme from *S. aureus*.

Although ciprofloxacin, trovafloxacin, levofloxacin, and sparfloxacin all stimulated DNA cleavage to a similar extent,

they differed widely in their potency. Under all conditions employed, trovafloxacin was the most potent quinolone examined. On the basis of preequilibrium experiments and cleavage site analysis, it appears that trovafloxacin forms DNA cleavage complexes more rapidly and at a greater number of sites than its less potent counterparts. An even more striking correlation was found between quinolone potency and the ability to inhibit DNA religation mediated by topoisomerase IV: the greater the cleavage enhancing potency of the drug, the slower the rate of religation.

The relationship between DNA cleavage potency and inhibition of religation was first suggested by the actions of ciprofloxacin against Gram-negative (*E. coli*) topoisomerase IV (*36*). While the effects of ciprofloxacin on religation were not as pronounced as in the present study, its potency against *E. coli* topoisomerase IV was several orders of magnitude lower than observed with the Gram-positive enzyme.

Eukaryotic type II topoisomerases are targeted by some members of the quinolone family (*36*, *57*, *58*, *60*−*69*). However, the potency of these antineoplastic quinolones against eukaryotic topoisomerase II is considerably lower than the potency of antibacterial quinolones against either *E. coli* or *S. aureus* topoisomerase IV (*36*, *45*, *57*, *61*). Furthermore, the mechanistic basis for the actions of quinolones against eukaryotic topoisomerase II appears to differ from those of quinolones against bacterial topoisomerase IV. Antineoplastic quinolones have little or no effect on DNA religation mediated by topoisomerase II and are presumed to act primarily by increasing the forward rate of DNA scission (*57*, *61*). Whether this change in drug mechanism reflects the decreased drug potency or an evolutionary difference in enzyme-quinolone interactions remains to be resolved.

Quinolone antibacterials appear to kill Gram-negative bacteria primarily by increasing levels of chromosomal cleavage mediated by DNA gyrase (*3*, *5*, *8*, *22*, *29*, *33*−*35*). The initial study on the actions of quinolones against topoisomerase IV in *E. coli* suggested that the ability of quinolones to inhibit enzyme activity might be of greater importance than their effects on DNA cleavage (*22*). However, a later genetic approach that utilized bacterial strains encoding drug-resistant forms of DNA gyrase provided strong evidence that quinolones kill cells by converting topoisomerase IV into a cellular toxin that generates chromosomal breaks (*37*).

Unfortunately, equivalent studies have yet to be carried out in Gram-positive bacteria, even though topoisomerase IV (as opposed to DNA gyrase) is the primary cellular target for most quinolones in these species (*3*, *21*, *24*−*26*, *30*, *33*, *34*, *52*). However, concentrations of trovafloxacin and ciprofloxacin required to kill *S. aureus* cells in culture approximate those required to stimulate DNA scission and reflect potency differences observed between the two quinolones in DNA cleavage assays (trovafloxacin was ~7-fold more potent than ciprofloxacin) (*39*). In addition, concentrations of trovafloxacin and ciprofloxacin required to inhibit topoisomerase IV-catalyzed DNA decatenation were nearly identical and were greater than 2 orders of magnitude beyond the clinical range of these drugs (*27*, *39*). Taken together, these results provide compelling circumstantial evidence that quinolone cytotoxicity against Gram-positive bacteria does not result from the inhibition of topoisomerase IV catalysis, but rather from the stimulation of topoisomerase IV-mediated DNA cleavage.

In summary, topoisomerase IV is the clinical target for a new generation of quinolone antibacterials that display enhanced activity against Gram-positive infections (*3*, *33*, *34*). Using a *S. aureus* model system, the present study provides insight into the basis for quinolone action and resistance, and further defines the mechanism by which these important drugs increase levels of DNA cleavage by Gram-positive topoisomerase IV.

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