ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1989, p. 1697–1703
0066-4804/89/101697-07$02.00/0

Vol. 33, No. 10

Use of In Vitro Topoisomerase II Assays for Studying Quinolone Antibacterial Agents

JOHN F. BARRETT,* THOMAS D. GOOTZ, PAUL R. McGUIRK, CATHY A. FARRELL, AND SHARON A. SOKOLOWSKI

Department of Immunology and Infectious Diseases, Pfizer Central Research, Pfizer, Inc., Eastern Point Road, Groton, Connecticut 06340

Received 24 March 1989/Accepted 7 July 1989

Several quinolones and antitumor compounds were tested as inhibitors of purified calf thymus topoisomerase II in unknotting, catenation, radiolabeled DNA cleavage, and quantitative nonradiolabeled cleavage assays. The antitumor agents VP-16 (demethylepipodophyllotoxin ethylio-β-D-glucoside) and ellipticine demonstrated drug-enhanced topoisomerase II DNA cleavage (the concentration of drug that induced 50% of the maximal DNA cleavage in the test system [CC_{50}]) at levels of ≤5 μg/ml. Nalidixic acid, norfloxacin, and oxolinic acid did not induce significant topoisomerase II DNA cleavage, whereas ciprofloxacin did induce some cleavage above background levels. CP-67,015, a new 6,8-difluoro-7-pyridyl 4-quinolone which possesses potent antibacterial activity, inhibited bacterial DNA gyrase at 0.125 μg/ml in a nonradioactive DNA cleavage assay. Unlike other quinolones characterized to date, CP-67,015 was shown to strongly enhance topoisomerase II-induced radiolabeled DNA cleavage with a CC_{50} of 33 μg/ml and demonstrated cleavage in a nonradiolabeled DNA cleavage assay with a CC_{50} of 73 μg/ml. The topoisomerase II-mediated cleavage of DNA by CP-67,015 is consistent with its reported clastogenic effect on DNA in cell culture and its positive mutagenic response in mouse lymphoma cells. In vitro topoisomerase II catalytic and cleavage assays are useful for gaining preliminary information concerning the possible interaction(s) of some quinolones with eucaryotic topoisomerase II which may relate directly to their safety (mutagenicity, clastogenicity, or both) in human and veterinary medicinal usage.

In vitro DNA cleavage mediated by mammalian topoisomerase II has been reported to occur (in the absence of drug) at high enzyme to DNA ratios (17, 19) and to be enhanced by antitumor compounds such as 4′-(9-acridinylamino)methanesulfon-*m*-aniside (m-AMSA), ellipticine, VP-16, and VM-26 (2, 4, 22, 24, 29, 30, 39). At the same time, these compounds have shown variable inhibitory effects on the catalytic activity of the enzyme (2, 4, 29). Since this class of drugs has also been associated with DNA breakage observed in cell culture, it has been suggested that enhanced topoisomerase II-mediated DNA cleavage is an important mechanism for the antitumor effects of these agents (4, 6, 20, 21, 24). Quinolone antibacterial agents are thought to exert their effects by a similar interference with bacterial DNA gyrase (6, 14, 23, 33), the bacterial homolog of eucaryotic topoisomerase II (15, 18, 21, 37). This class of antibacterial agents has recently been introduced into clinical use (1, 38). Since interaction with topoisomerase II is central to the activity of both quinolones and antitumor agents, it is of interest from a safety standpoint (5, 12, 13) to determine the effects of quinolone antibacterial agents on mammalian topoisomerase II, especially in light of the recent controversy over the mechanism of action of 4-quinolone antibacterial agents as to whether quinolones bind to DNA, gyrase, or the ternary complex of DNA gyrase (7, 11, 13, 32, 36).

Several different assays have been developed by others (16–19, 21, 22, 27–29, 37, 40) for detecting the effects of antitumor agents on mammalian topoisomerase II. These have included monitoring the catalytic unknotting and catenation activities of the enzyme on DNA (4, 16, 17, 24, 30), their ability to induce DNA cleavage in the presence of drug using [^{32}P]ATP-labeled plasmid as substrate (4, 24, 34, 40), and filter-binding assays that measure DNA breakage (29). Liu and colleagues (4, 24) have also examined the ability of antitumor compounds to mediate DNA cleavage in a nonradiolabeled assay, although they have not quantitated such results. The [^{32}P]ATP-radiolabeled DNA cleavage assay is a sensitive method of detecting drug-induced DNA cleavage by topoisomerase II and can be used to map drug-specific cleavage sites (4, 24, 34, 40). We describe the use of four assays, including a quantitative nonradiolabeled DNA cleavage assay, for determining the catalytic and DNA cleavage effects of some quinolones on eucaryotic (calf thymus) topoisomerase II. One quinolone, CP-67,015, demonstrated atypical effects against eucaryotic topoisomerase II which heretofore was thought not to be a target for quinolone antibacterial agents.

MATERIALS AND METHODS

Enzymes, nucleic acids, and chemicals. Knotted P4 DNA was purified from P4 tailless capsids as described elsewhere (16). Proteinase K was purchased from Boehringer-Mannheim Biochemicals (Indianapolis, Ind.). pBR322 DNA was purchased from Pharmacia Fine Chemicals (Piscataway, N.J.). Norfloxacin was kindly provided by Merck Pharmaceutical Co. (Rahway, N.J.). Nalidixic acid, oxolinic acid, ellipticine, adriamycin, and tRNA type XXI were purchased from Sigma Chemical Co. (St. Louis, Mo.). VP-16 (demethylepipodophyllotoxin ethylide-β-D-glucoside) was kindly provided by the National Cancer Institute (Bethesda, Md.). Ciprofloxacin was synthesized by the procedure described in U.S. patent 4,670,444 (June 1987). CP-67,015 (see Fig. 1) was synthesized at Pfizer Central Research by the procedure described in U.S. patent 4,636,506 (January 1987). All other chemicals were of reagent grade.

Densitometric analyses. Densitometric analyses of photo-

* Corresponding author.

graphs of ethidium bromide-stained (1.25 μg/ml) DNA agarose gels visualized by transillumination with UV light were done in the reflective mode (cool white light) on a video densitometer (model 620; Bio-Rad Laboratories, Richmond, Calif.).

Topoisomerase II purification. Topoisomerase II was purified from the thymus of freshly slaughtered calves by the procedure of either Halligan et al. (9) or Schomburg and Grosse (31). The procedure of Schomburg and Grosse (31) resulted in the isolation of the predominantly unproteolyzed (1.1×10^6 U/mg) and topoisomerase I-free form of topoisomerase II (31), enabling the recovery of a higher-activity enzyme with both catenation activity and sufficient activity to generate drug-free, nonradiolabeled cleavage of pBR322. Topoisomerase II purified by the procedure of Halligan et al. (9) resulted in the isolation of the predominantly proteolyzed form of the enzyme (0.9×10^5 U/mg) with some minor topoisomerase I contamination (which precluded its use in the cold cleavage assay). A unit of eucaryotic topoisomerase II activity was defined as the amount of enzyme required to completely catenate 0.38 μg of pBR322 DNA in 60 min at 37°C under the reaction conditions described below.

DNA gyrase purification. DNA gyrase subunits A (1.9×10^4 U/mg) and B (0.54×10^4 U/mg) were purified from *Escherichia coli* overproducer strains (provided by M. Gellert) by the method of Mizuuchi et al. (23). Gyrase activity of 1 U was defined as the amount of enzyme necessary to completely supercoil 0.3 μg of pBR322 in 30 min at 37°C; 1 U of holoenzyme used in the gyrase assays was composed of 1 U of activity of subunit A and 1 U of activity of subunit B (assayed independently in the excess of the complementary subunit).

DNA gyrase cleavage assay. The general protocol for the DNA gyrase cleavage assay was modified from those of Gellert et al. (8) and Sugino et al. (33). Approximately 2 U of reconstituted DNA gyrase holoenzyme was incubated in 35 mM Tris hydrochloride (pH 7.5)–10 mM $MgCl_2$–5 mM spermidine hydrochloride–5 mM dithiothreitol–0.2 mM ATP–50 μg of bovine serum albumin per ml–100 μg of *E. coli* tRNA per ml–0.3 μg of pBR322 DNA in 30-μl reactions containing the appropriate concentration of drug being tested (usually twofold serial dilutions from 500 down to 0.39 μg/ml) at 25°C. After 60 min, the reaction was stopped by the addition of sodium dodecyl sulfate to a final concentration of 0.2% sodium dodecyl sulfate, followed by deproteination by the addition of proteinase K at 90 μg/ml and incubation for 30 min at 37°C. After deproteination, the reaction mix was mixed with 1 μl of tracking dye (50% glycerol [wt/vol] and 0.125% bromophenol blue), loaded onto a 0.7% TBE-agarose gel (89 mM Tris, 89 mM boric acid, 2 mM EDTA [pH 8.3]), and electrophoresed. The denaturing of the drug-gyrase-DNA complex led to the appearance of linear DNA that was detected as a single band between relaxed and supercoiled DNAs after separation of products by electrophoresis, staining with ethidium bromide, and photography on a UV light transilluminator. The DNA cleavage endpoint value was the minimum amount of drug to induce detectable cleavage of the supercoiled pBR322 substrate to the linear form.

Radiolabeled cleavage assay. The radiolabeled cleavage assay was done by using [^{32}P]ATP-end-labeled pBR322 DNA as substrate by the procedure of Tewey et al. (34), with minor modifications. Modifications included the use of 2 mM ATP and between 65 and 780 ng of topoisomerase II (60 to 70 U) per reaction mix, depending on the method of purification (Schomburg and Grosse [31] and Halligan et al. [9], respectively). The procedure for end-labeling was done as described elsewhere (24). Briefly, *Eco*RI-digested pBR322 DNA was labeled with Klenow fragment, [α-^{32}P]ATP, and nonradiolabeled dTTP; and then it was cleaved with *Hin*dIII to generate a single-end-labeled fragment of 4,332 base pairs.

Catenation assay. The catenation assay was done by using a modified version of the procedure of Schomburg and Grosse (31). Modifications included the following changes in the reaction cocktail: 50 mM Tris hydrochloride (pH 8.0), 25 mM NaCl, 10 μg of bovine serum albumin per ml, 2 mM dithiothreitol (in place of 2-mercaptoethanol), and 0.38 μg of pBR322 DNA per 25-μl reaction mix. The topoisomerase II purified by the procedure of Schomburg and Grosse (31) was used in this assay at 4.3 ng/25-μl reaction (4 to 5 U). Reactions were incubated for 1 h at 37°C and stopped by the addition of sodium dodecyl sulfate to 0.9%. Samples were deproteinated by incubation with proteinase K (final concentration, 0.8 mg/ml) for 45 min at 50°C and then electrophoresed in 0.4% TBE-agarose gels and stained with ethidium bromide. The concentration of drug to cause 50% inhibition of activity (IC_{50}) was determined by densitometric analyses of the formation of product (catenated DNA) in the presence of the dilution of compound being tested versus that in the drug-free control enzyme reaction. Uncatenated DNA was measured as arbitrary units in the reflective (cool white light) mode versus time from the photograph of the ethidium bromide-stained gel.

P4 unknotting assay. The P4 unknotting assay was a modified version of the unknotting procedure of Liu et al. (16). Modifications included the following changes. The amount of topoisomerase II added to the reaction mix was 1 U (which was the amount of enzyme required to unknot 50% of the P4 knotted DNA in 30 min at 37°C); all drug dilutions were done in dimethyl sulfoxide (final concentration, 1.3%); and after incubation for 30 min at 37°C, the reactions were terminated by placing the reaction tubes in an ice water bath, electrophoresed in 0.7% TAE-agarose gels (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA [pH 7.2]), and stained with ethidium bromide. Densitometric quantitation of the unknotted DNA was expressed as a percentage of the completely unknotted (~60-min) sample.

Nonradiolabeled cleavage assay. The nonradiolabeled cleavage assay was done by the method of Farrell and Barrett (C. A. Farrell and J. F. Barrett, manuscript in preparation). Essentially, calf thymus topoisomerase II, which was purified by the procedure of the Schomburg and Grosse (31), was used to establish a nonradiolabeled topoisomerase II DNA cleavage assay. A total of 0.5 μg of supercoiled pBR322 DNA, 22 ng of topoisomerase II (22 to 24 U) purified by the procedure of Schomburg and Grosse (31), and various dilutions of the compound being tested were combined in a total reaction volume of 25 μl (10 mM Tris [pH 7.5], 10 mM $MgCl_2$, 10 mM KCl [34]) and were incubated for 1 h at 37°C. The reaction was stopped by the addition of sodium dodecyl sulfate to 0.1%, followed by deproteination with proteinase K at 0.8 mg/ml for 45 min at 50°C. Samples were electrophoresed in a 1% TBE-agarose gel and stained with ethidium bromide, and the amount of cleaved, linear DNA was quantitated by densitometry to determine the concentration of drug that induced 50% of the maximal DNA cleavage in the test system (CC_{50}) for inhibition by a dilution of the compound being tested relative to that of a control compound.

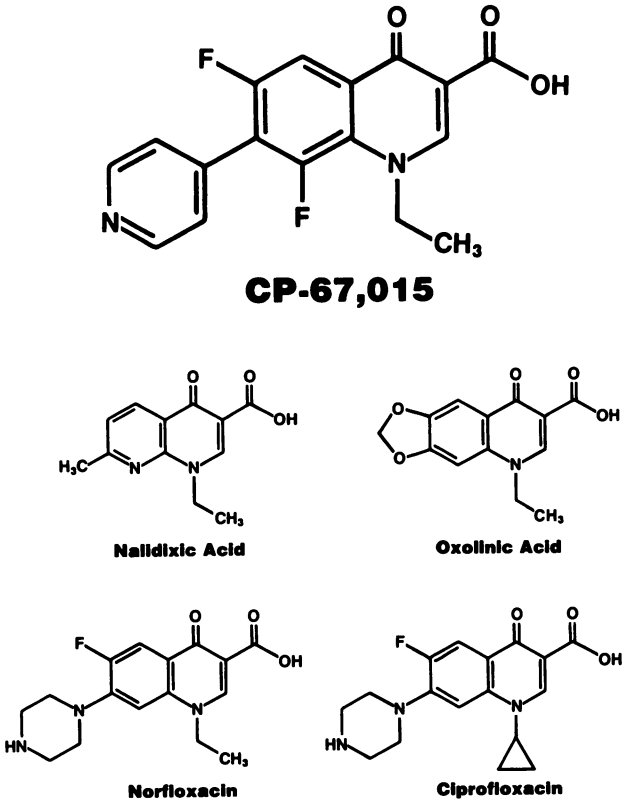

FIG. 1. Structures of the quinolones CP-67,015, nalidixic acid, oxolinic acid, norfloxacin, and ciprofloxacin.

RESULTS

P4 unknotting and catenation assays. The antitumor agents (adriamycin, ellipticine, and VP-16) and the DNA gyrase inhibitors (nalidixic acid, oxolinic acid, norfloxacin, ciprofloxacin, and CP-67,015; Fig. 1) were studied for their effects on the unknotting and catenation activities of topoisomerase II purified from calf thymus. Initial time course assays for both enzyme activities were run in the absence of drug in order to define the parameters that provided linear reaction kinetics. As shown in Fig. 2 and 3, the time course studies of these catalytic assays identified linear regions of the reaction where a 50% completion point could be determined from densitometric plots of photographs of ethidium bromide-stained DNA bands of unknotting and catenation, respectively. Initial steady-state kinetics were exhibited over at least the first 30 min of the unknotting reaction, with approximately all substrate being converted to product in 60 min (Fig. 2). Likewise, with catenation, initial catalysis was approximately linear for at least the first 30 min (Fig. 3). The parameters of time and enzyme activity that defined an IC_{50} were used for determining the effect of test drugs on P4 unknotting and catenation of pBR322. The inhibitory activity of ellipticine was compared with those of three of the quinolones tested in the P4 unknotting assay (Fig. 4). The degree of unknotting obtained in the presence of each drug was compared with that of the drug-free control under the conditions of the assay. As shown in Fig. 4 and Table 1, adriamycin and ellipticine were highly inhibitory in the unknotting assay, with IC_{50}s of 0.5 and 4.9 μg/ml, respectively, consistent with reports in the literature (35). The quinolones were at least 20-fold less inhibitory (Table 1). A

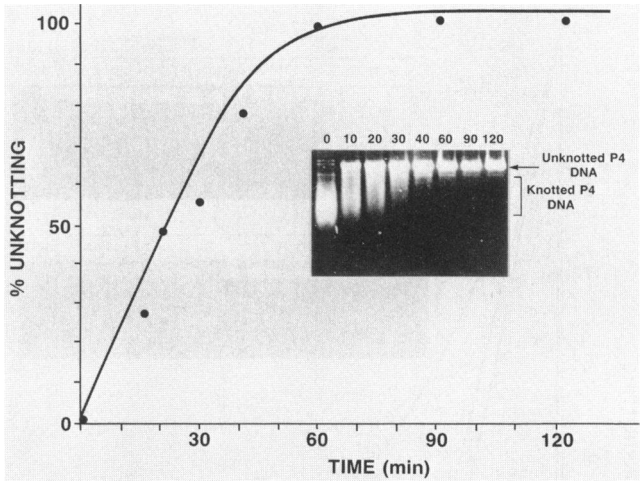

FIG. 2. Unknotting of P4 head DNA. Time course study illustrating the unknotting of P4 head DNA to its simple circular form by calf thymus topoisomerase II. The inset shows a photograph of the ethidium-bromide stained DNA agarose gel (after electrophoresis) time course study, indicating the relative positions of the unknotted and knotted P4 DNAs at the indicated times (in minutes) after electrophoresis.

similar pattern was observed in the catenation reaction (Fig. 5 and 6). Adriamycin and ellipticine were highly inhibitory for topoisomerase II catenation activity when pBR322 was used as the substrate. VP-16 was significantly less inhibitory than the other antitumor agents (IC_{50}, 70 μg/ml), while the quinolones tested were not inhibitory at levels of less than ~700 μg/ml (Table 1), with the exceptions of CP-67,015 (IC_{50}, 265 μg/ml) and ciprofloxacin (IC_{50}, 325 μg/ml).

Topoisomerase II-mediated cleavage assays. Since the quinolones tested demonstrated little inhibition of the catalytic

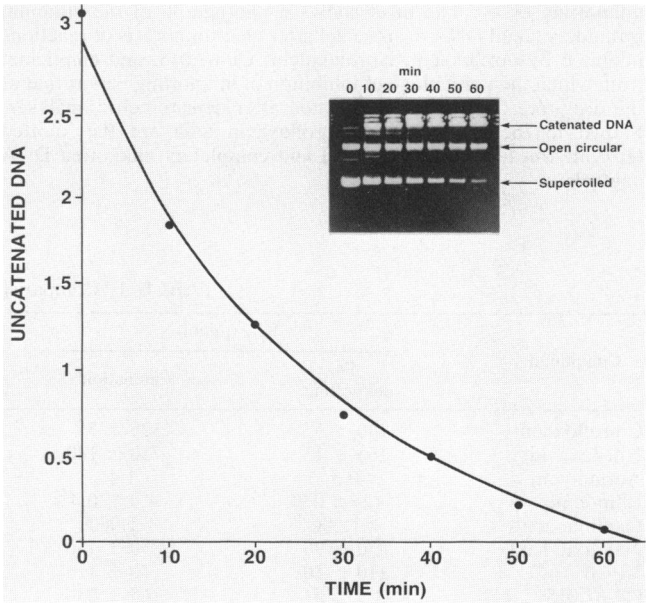

FIG. 3. Time course of DNA catenation by calf thymus topoisomerase II. The inset shows a photograph of the ethidium bromide-stained DNA agarose gel after electrophoresis from which the time course of DNA catenation by the topoisomerase II was derived after densitometric analysis.

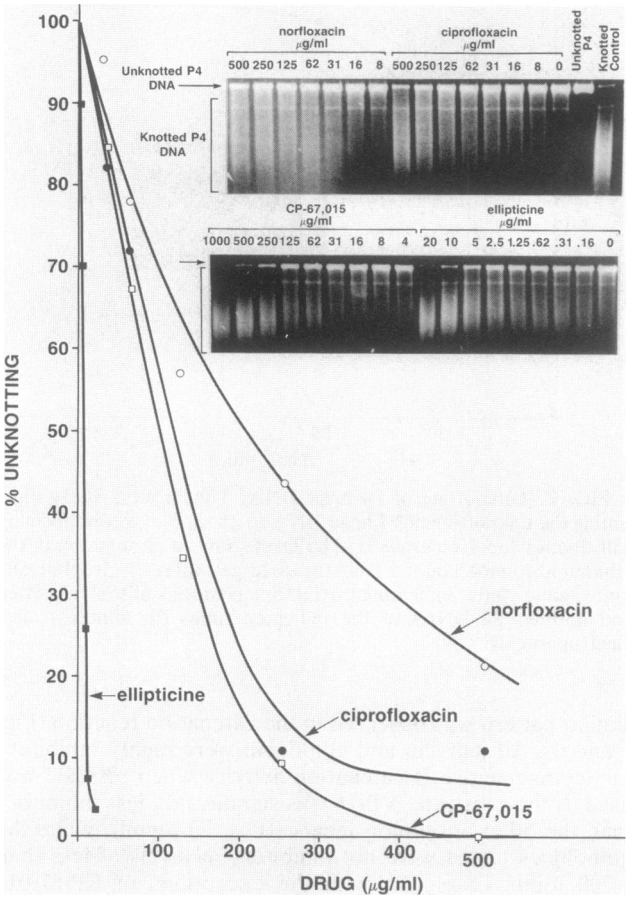

FIG. 4. Comparative inhibitory effects of different quinolones in the P4 unknotting assay. Norfloxacin, ciprofloxacin, and CP-67,015 are representative of the quinolones tested that show the need for much higher drug levels compared with the level of ellipticine (representative of the antitumor compounds) in testing in the P4 unknotting assay. The inset shows a photograph of the ethidium bromide-stained DNA agarose gel after electrophoresis of reactions inhibited by norfloxacin, ciprofloxacin, CP-67,015, and ellipticine, from which the percentage of inhibition of unknotting versus that of the drug-free control was calculated after densitometric analyses. Shown to the right of the ciprofloxacin data are the knotted (enzyme-free P4 DNA substrate) and completely unknotted DNA controls.

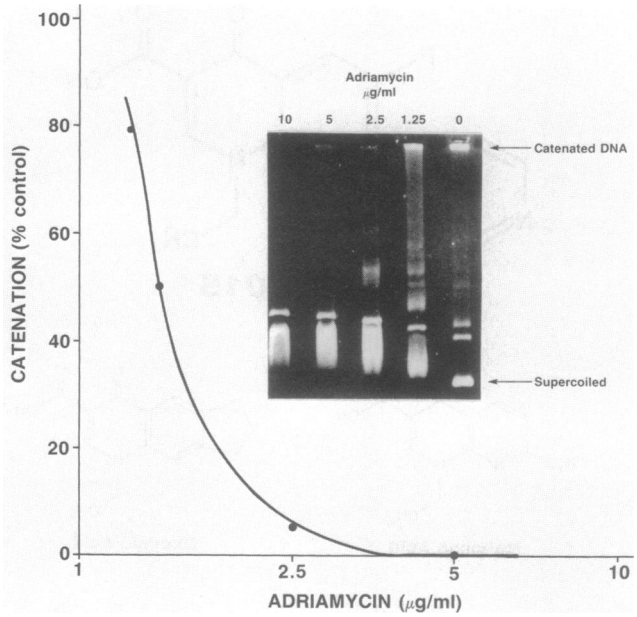

FIG. 5. Inhibition of catenation activity of calf thymus topoisomerase II by adriamycin. The adriamycin-concentration-dependent inhibition of catenation of pBR322 by mammalian topoisomerase II is shown as a percentage of that of the drug-free control. The inset shows a photograph of the ethidium bromide-stained DNA agarose gel after electrophoresis of reactions inhibited by adriamycin.

activity of topoisomerase II, a radiolabeled cleavage assay was used to determine whether they induced DNA cleavage in the presence of topoisomerase II. [^{32}P]ATP-end-labeled pBR322 was used for this purpose. Figure 7 shows photographs of autoradiographs of the cleavage products from the labeled DNA-drug-enzyme complex after electrophoresis. As expected, the antitumor agent VP-16 showed significant concentration-dependent enhancement of topoisomerase II-mediated cleavage of the labeled pBR322 fragment, consistent with the report from Chen et al. (4). CP-67,015 also induced significant cleavage with topoisomerase II at an eightfold higher concentration (CC_{50}, 33 μg/ml), but was more potent in this regard than any other quinolone tested, although ciprofloxacin demonstrated radiolabeled DNA cleavage with a CC_{50} of 120 μg/ml (Table 1), indicating that it was approximately fourfold less potent than CP-67,015.

TABLE 1. Comparison of topoisomerase assays[a]

Compound	IC_{50} (μg/ml)		Bacterial gyrase cleavage endpoint (μg/ml)[b]	CC_{50} (μg/ml)	
	P4 unknotting[c]	Catenation[d]		Radiolabeled DNA cleavage	Nonradiolabeled DNA cleavage
Ciprofloxacin	140 ± 5	325 ± 5	0.20	120	>1,000
Norfloxacin	165 ± 15	730 ± 180	0.78	>1,000	>1,000
Adriamycin	0.5	1.4	ND[e]	ND	ND
Ellipticine	4.9 ± 0.7	4.0 ± 0.4	ND	~0.125	ND
Oxolinic acid	>1,000	>1,000	6.25	>1,000	>1,000
Nalidixic acid	850 ± 90	1,850 ± 150	25	>1,000	>2,000
VP-16	110 ± 10	70 ± 11	>500	4.5	7.5 ± 4.5
CP-67,015	92.5 ± 5	265 ± 23	0.125	33	73 ± 17

[a] Topoisomerase II was isolated from calf thymus by the procedure of Schomburg and Grosse (31).
[b] The bacterial gyrase cleavage endpoint was the minimum amount of drug to induce detectable cleavage.
[c] The P4 unknotting assay was modified from that of Liu et al. (16).
[d] The catenation assay was modified from that of Schomburg and Grosse (31).
[e] ND, Not determined because of intercalation of the compound into the substrate.

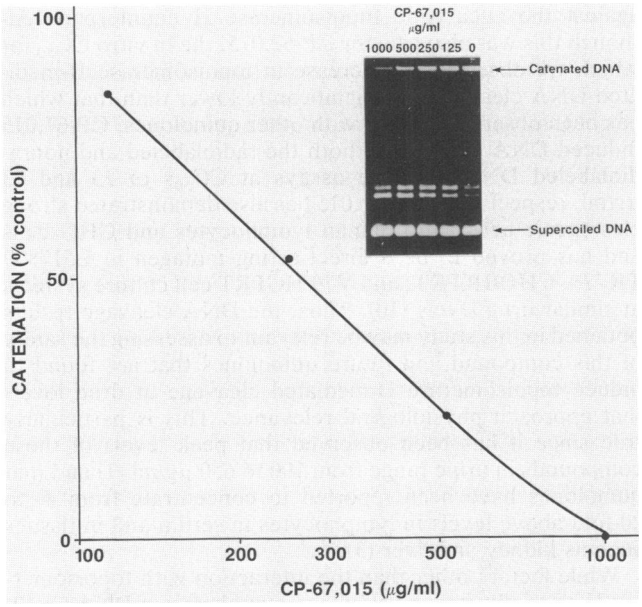

FIG. 6. Inhibition of catenation activity of mammalian topoisomerase II by CP-67,015. Concentration-dependent inhibition of catenation by CP-67,015. The inset shows a photograph of the ethidium bromide-stained DNA agarose gel after electrophoresis, from which the graphical data were derived after densitometric analysis.

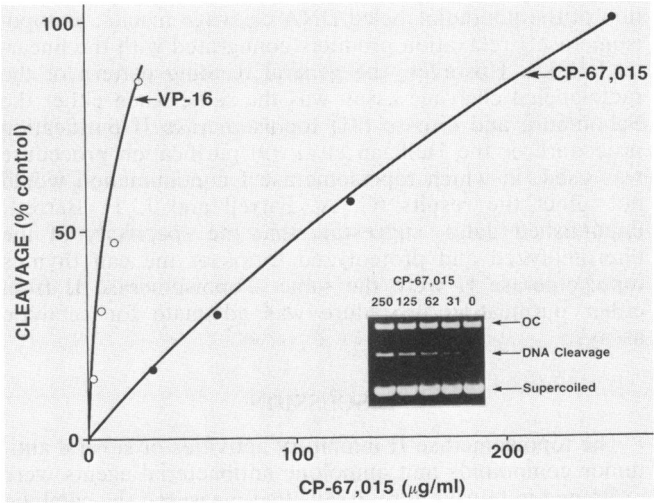

FIG. 8. Drug-induced DNA cleavage in a nonradiolabeled cleavage assay. Cleavage of supercoiled pBR322 DNA to the linear form by calf thymus topoisomerase II in the presence of VP-16 or CP-67,015 is shown as a percentage of that of the drug-free control. The inset shows a photograph of the ethidium bromide-stained DNA agarose gel after electrophoresis of the reaction mix containing various concentrations (in micromolar) of CP-67,015 (with the relative migration of the open circular, cleaved, and linear DNA and the supercoiled DNA indicated).

The quinolone class of drugs appeared to have a different pattern of banding on the autoradiographs in comparison with those of the antitumor drugs (Fig. 7), suggesting that there are specific, preferred cleavage sites, as has been reported for antitumor agents (4, 24, 34, 40). The enhanced DNA cleavage activity of CP-67,015 was also observed in the topoisomerase II nonradiolabeled DNA cleavage assay with nonradiolabeled pBR322 used as the substrate (Fig. 8). No other quinolones included in this study stimulated DNA cleavage above background levels in the nonradiolabeled DNA cleavage assay when tested at concentrations of up to 1,000 μg/ml (Table 1).

Utilization of this nonradiolabeled DNA cleavage assay was possible because of the higher specific activity of the topoisomerase II preparation purified by the procedure of Schomburg and Grosse (31) relative to the specific activity of the topoisomerase II with topoisomerase I contamination. In our hands, somewhat more topoisomerase I contamination was found in the topoisomerase II preparation purified by the procedure of Halligan et al. (9) than in the topoisomerase II purified by the procedure of Schomburg and Grosse (31). At the level of enzyme needed to observe cleavage of linear DNA, the presence of the relaxation activity from the contaminating topoisomerase I interfered with the quantita-

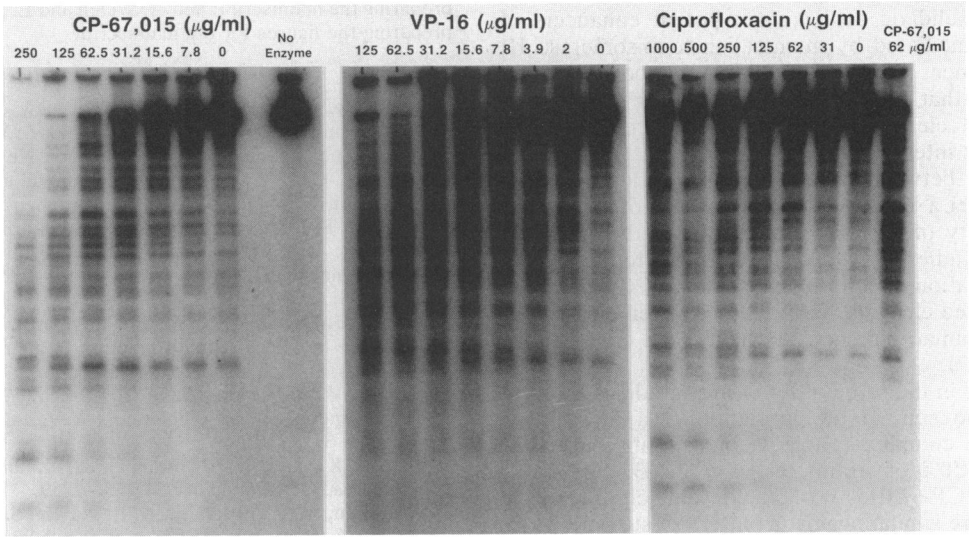

FIG. 7. Inhibition of mammalian topoisomerase II by quinolones in the radiolabeled cleavage assay. Banding patterns of CP-67,015, VP-16, and ciprofloxacin are shown in the analyses of the autoradiograph (exposed for 72 h) of the drug concentration-dependent cleavage of [^{32}P]ATP-single-end-labeled pBR322 by calf thymus topoisomerase II. Shown between the CP-67,015 and VP-16 cleavage data is the enzyme-free control of unhydrolyzed [^{32}P]ATP-single-end-labeled pBR322 DNA.

tion of the nonradiolabeled DNA cleavage results, as topoisomerase I relaxation products comigrated with the linearized DNA. However, the general banding pattern of the radiolabeled cleavage assay was the same when either the Schomburg and Grosse (31) topoisomerase II purification procedure or the Halligan et al. (9) purification procedure was used, in which topoisomerase I contamination would not affect the results (C. A. Farrell and J. F. Barrett, unpublished data), suggesting that the specificity of the unproteolyzed and proteolyzed forms of the calf thymus topoisomerase II were the same. Topoisomerase II from either purification procedure was adequate for catalytic assays.

DISCUSSION

The topoisomerase II inhibitory activities of several antitumor compounds and quinolone antibacterial agents were examined in four in vitro assays that measured the catalytic or DNA cleavage activities of a mammalian topoisomerase II enzyme, including a quantitative assay that assessed the cleavage of unlabeled DNA substrate. Other investigators (2, 4, 24, 30, 34, 35) have shown that antitumor compounds such as adriamycin, ellipticine, *m*-AMSA, and VP-16 are inhibitory to the catalytic activity of topoisomerase II and enhance DNA cleavage by topoisomerase II. The assays used in this study confirmed this. The effects of quinolone antibacterial agents on mammalian topoisomerase II have been less well studied, raising questions about safety with regard to reports of the binding of 4-quinolones to DNA rather than gyrase (32, 36). In agreement with our results, Hussy et al. (13) did not find quinolones to be very inhibitory to topoisomerase II catenation activity, with K_i values ranging from 150 to 1,000 μg/ml for ciprofloxacin, oxolinic acid, norfloxacin, and nalidixic acid. Likewise, Riou et al. (30) have found that nalidixic acid and pefloxacin are poor inhibitors of catenation and decatenation activities of a trypanosome topoisomerase II. Consistent with these results were our minimum drug concentrations for stimulating topoisomerase II-mediated cleavage in the "hot" cleavage assay (Farrell and Barrett, unpublished data).

Our findings indicate that ciprofloxacin, norfloxacin, oxolinic acid, and nalidixic acid are not potent enhancers of DNA cleavage mediated by mammalian topoisomerase II; and the significance, if any, of the lower drug concentration of ciprofloxacin that was observed to enhance cleavage in the radiolabeled cleavage assay (Table 1) could not be determined. It is interesting that Oomori et al. (25) found a good correlation between the cytotoxicity of quinolones in vitro against HeLa cells with their ability to inhibit the relaxation activity of topoisomerase II purified from these cells. In those studies (25), ciprofloxacin was found to give an IC_{50} for relaxation inhibition similar to the CC_{50} obtained in our radiolabeled cleavage assay. Other adverse effects of quinolones on human lymphocytes have been observed by Forsgren et al. (7).

CP-67,015 was in the same order of magnitude as norfloxacin and ciprofloxacin in the formation of a measurable gyrase-cleavable complex (Table 1); and antibacterial potency MICs for 50% of strains tested of 0.063, 0.031, and 0.063 μg/ml for CP-67,015, norfloxacin, and ciprofloxacin, respectively, were similar against a battery of susceptible *E. coli* test strains. As reported elsewhere (13, 30), we found that the enhancement of topoisomerase II-mediated DNA cleavage activity by quinolones against procaryotic DNA gyrase was approximately 100-fold more potent than it was against the eucaryotic topoisomerase II counterpart. Although this was also true for CP-67,015, the in vitro CC_{50} for which we observed an increase in topoisomerase II-mediated DNA cleavage was significantly lower than that which has been observed to date with other quinolones. CP-67,015 induced DNA cleavage in both the radiolabeled and nonradiolabeled DNA cleavage assays at CC_{50}s of 33 and 73 μg/ml, respectively. CP-67,015 has also demonstrated strong clastogenic activity in human lymphocytes and CHO cells and has proved to be a direct-acting mutagen in L5178Y/TK$^{+/-}$, CHO/H6PRT, and V79/HGPRT cell culture systems at similar drug levels (10). Thus, the DNA cleavage results obtained in this study may be relevant to assessing the safety of this compound and future quinolones that are found to induce topoisomerase II-mediated cleavage at drug levels that approach physiological relevance. This is particularly true since it has been observed that peak levels of these compounds in urine range from 100 to 650 μg/ml (1) and that quinolones have been reported to concentrate from 4- to 20-fold above levels in lymphocytes in serum and in tissues such as kidney and liver (3).

While factors other than the interaction with topoisomerase II may influence the effects of quinolones on DNA (7, 12, 13, 26, 32, 36), the in vitro cleavage and catalytic assays used in this study have merit in evaluating the selectivities and specificities of these agents against bacterial gyrase. In addition, the stimulation of topoisomerase II-mediated DNA cleavage by quinolones such as CP-67,015 (10) should be viewed with some concern in light of the positive in vivo cytogenetic results observed with CP-67,015, and until the in vivo relevance of such data is determined (5, 10, 12, 13, 26).

ACKNOWLEDGMENTS

We thank K. Drlica, M. Gellert, L. F. Liu, and K. M. Tewey for assistance in starting up and troubleshooting the assays; L. F. Liu for kindly providing the P4 phage and the *E. coli* host and for determining the titers of *E. coli* strains, as well as for details concerning the purification of the P4 DNA; M. Gellert for providing the *E. coli* strains that overproduced gyrase A and B subunits; R. Isaacson for helpful strategy discussions and critical review of the manuscript; K. Brighty, J. Retsema, and A. Redborg for critical review and suggestions with the manuscript; S. Haskell with help in preparing the manuscript; and G. Welch and E. Bellefleur for help in preparing the figures for the manuscript.

LITERATURE CITED

1. **Andriole, V. T.** 1988. Clinical overview of the newer 4-quinolone antibacterial agents, p. 155–200. *In* V. T. Andriole (ed.), The quinolones. Academic Press, Inc., New York.
2. **Auclair, C.** 1987. Multimodal action of antitumor agents on DNA: the ellipticine series. Arch. Biochem. Biophys. **259**:1–14.
3. **Bergan, T.** 1988. Pharmacokinetics of fluorinated quinolones, p. 119–154. *In* V. T. Andriole (ed.), The quinolones. Academic Press, Inc., New York.
4. **Chen, G. L., L. Yang, T. C. Rowe, B. D. Halligan, K. M. Tewey, and L. F. Liu.** 1984. Nonintercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian topoisomerase II. J. Biol. Chem. **259**:13560–13566.
5. **Christ, W., T. Lehnert, and U. Ulbrich.** 1988. Specific toxicologic aspects of the quinolones. Rev. Infect. Dis. **10**(Suppl. 1):S141–S149.
6. **Drlica, K., and R. J. Franco.** 1988. Inhibitors of DNA topoisomerases. Biochemistry **27**:2253–2259.
7. **Forsgren, A., A. Bredberg, A. B. Pardee, S. F. Schlossman, and T. F. Tedder.** 1987. Effects of ciprofloxacin on eukaryotic pyrimidine nucleotide biosynthesis and cell growth. Antimicrob. Agent Chemother. **31**:774–779.
8. **Gellert, M., K. Mizuuchi, M. H. O'Dea, T. Itoh, and J.-I. Tomizawa.** 1977. Nalidixic acid resistance: a second genetic

character involved in DNA gyrase activity. Proc. Natl. Acad. Sci. USA 74:4772–4776.
9. **Halligan, B. D., K. A. Edwards, and L. F. Liu.** 1985. Purification and characterization of a type II DNA topoisomerase from bovine calf thymus. J. Biol. Chem. **260**:2475–2482.
10. **Holden, H. E., J. F. Barrett, C. M. Huntington, P. A. Muehlbauer, and M. G. Wahrenburg.** 1989. Genetic profile of a nalidixic acid analog: a model for the mechanism of sister chromatid exchange induction. Environ. Mol. Mutagen. **13**: 238–252.
11. **Hooper, D. C., and J. S. Wolfson.** 1988. Mode of action of the quinolone antimicrobial agent. Rev. Infect. Dis. **10**(Suppl. 1): S14–S21.
12. **Hosomi, J., A. Maeda, Y. Oomori, T. Irikura, and T. Yokota.** 1988. Mutagenicity of norfloxacin and AM-833 in bacteria and mammalian cells. Rev. Infect. Dis. **10**(Suppl. 1):S148–S149.
13. **Hussy, P., G. Maass, B. Tummler, F. Grosse, and U. Schomburg.** 1986. Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase α primase, topoisomerases I and II, and growth of mammalian lymphoblasts. Antimicrob. Agents Chemother. **29**:1073–1078.
14. **Lilley, D. M. J.** 1986. DNA topoisomerase inhibitors as antimicrobial agents. Biochem. Soc. Trans. **14**:489–493.
15. **Liu, L. F.** 1984. DNA topoisomerases—enzymes that catalyze the breaking and rejoining of DNA. Crit. Rev. Biochem. **15**: 1–24.
16. **Liu, L. F., J. L. Davis, and R. Calendar.** 1981. Novel topologically knotted DNA from bacteriophage P4 capsids: studies with DNA topoisomerases. Nucleic Acid. Res. **9**:3979–3989.
17. **Liu, L. F., B. D. Halligan, E. M. Nelson, T. C. Rowe, G. L. Chen, and K. M. Tewey.** 1983. Breakage and reunion of DNA helix by mammalian DNA topoisomerase II, p. 43–53. *In* N. R. Cozzarelli (ed.), Mechanisms of DNA replication and recombination. Alan R. Liss, Inc., New York.
18. **Liu, L. F., C.-C. Liu, and B. M. Alberts.** 1980. Type II DNA topoisomerases: enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. Cell **19**:697–707.
19. **Liu, L. F., T. C. Rowe, L. Yang, K. M. Tewey, and G. L. Chen.** 1983. Cleavage of DNA by mammalian DNA topoisomerase II. J. Biol. Chem. **258**:15365–15370.
20. **Lock, R. B., and W. E. Ross.** 1987. DNA topoisomerases in cancer therapy. Anti-Cancer Drug Design **2**:151–164.
21. **Maxwell, A., and M. Gellert.** 1986. Mechanistic aspects of DNA topoisomerases. Adv. Protein Chem. **38**:69–107.
22. **Minford, J., Y. Pommier, J. Filipski, K. W. Kohn, D. Kerrigan, M. Mattern, S. Michaels, R. Schwartz, and L. A. Zwelling.** 1986. Isolation of intercalator-dependent protein-linked DNA strand cleavage activity from cell nuclei and identification as topoisomerase II. Biochemistry **25**:9–16.
23. **Mizuuchi, K., M. H. O'Dea, and M. Gellert.** 1978. DNA gyrase: subunit structure and ATPase activity of the purified enzyme. Proc. Natl. Acad. Sci. USA **75**:5960–5963.
24. **Nelson, E. M., K. M. Tewey, and L. F. Liu.** 1984. Mechanism of antitumor drug action: poisoning of mammalian DNA topoisomerase II on DNA by 4′-(9-acridinylamino)methanesulfon-m-anisidide. Proc. Natl. Acad. Sci. USA **81**:1361–1365.
25. **Oomori, Y., Y. Tokutaro, H. Aoyama, K. Hirai, S. Suzue, and T. Yokota.** 1988. Effects of fleroxacin on HeLa cell functions and topoisomerase II. J. Antimicrob. Chemother. **22**(Suppl. D): 91–97.
26. **Phillips, I.** 1987. Bacterial mutagenicity and the 4-quinolones. J. Antimicrob. Chemother. **20**:771–773.
27. **Pommier, Y., M. R. Mattern, R. E. Schwartz, and L. A. Zwelling.** 1984. Absence of swiveling at sites of intercalator-induced protein-associated deoxyribonucleic acid strand breaks in mammalian cell nucleoids. Biochemistry **23**:2922–2927.
28. **Pommier, Y., M. R. Mattern, R. E. Schwartz, L. A. Zwelling, and K. W. Kohn.** 1984. Changes in deoxyribonucleic acid linking number due to treatment of 4′-(9-acridinylamino)methanesulfon-m-anisidide. Biochemistry **23**:2927–2932.
29. **Pommier, Y., J. K. Minford, R. E. Schwartz, L. A. Zwelling, and K. W. Kohn.** 1985. Effects of the DNA intercalators 4′-(9-acridinylamino)methanesulfon-m-anisidide and 2-methyl-9-hydroxyellipticinium on topoisomerase II mediated strand cleavage and strand passage. Biochemistry **24**:6410–6416.
30. **Riou, G., S. Douc-Rasy, and A. Kayser.** 1986. Inhibitors of trypanosome topoisomerases. Biochem. Soc. Trans. **14**:496–499.
31. **Schomburg, U., and F. Grosse.** 1986. Purification and characterization of DNA topoisomerase II from calf thymus associated with polypeptides of 175 and 150 kDa. Eur. J. Biochem. **160**:451–457.
32. **Shen, L. L., and A. G. Pernet.** 1985. Mechanism of inhibition of DNA gyrase by analogues of nalidixic acid: the target of the drug is DNA. Proc. Natl. Acad. Sci. USA **82**:307–311.
33. **Sugino, A., C. L. Peebles, K. N. Kruezer, and N. R. Cozzarelli.** 1977. Mechanism of action of nalidixic acid: purification of *Escherichia coli* in nalA gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. USA **74**:4767–4771.
34. **Tewey, K. M., G. L. Chen, E. M. Nelson, and L. F. Liu.** 1984. Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian topoisomerase II. J. Biol. Chem. **259**:9182–9187.
35. **Tewey, K. M., T. C. Rowe, L. Yang, B. D. Halligan, and L. F. Liu.** 1984. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science **226**:466–468.
36. **Tornaletti, S., and A. M. Pedrini.** 1988. Studies on the interaction of 4-quinolones with DNA by DNA unwinding experiments. Biochem. Biophys. Acta **949**:279–287.
37. **Wang, J. C.** 1985. DNA topoisomerases. Annu. Rev. Biochem. **54**:665–697.
38. **Webster, A., and H. Gaya.** 1988. Quinolones in the treatment of serious infections. Rev. Infect. Dis. **10**(Suppl. 1):S225–S233.
39. **Woynarowski, J. H., R. D. Sigmund, and T. A. Beerman.** 1988. Topoisomerase II-mediated lesions in nascent DNA: comparison of the effects of epipodophyllotoxin derivatives, VM-26 and VP-16, and 9-anilinoacridine derivatives, m-AMSA and o-AMSA. Biochem. Biophys. Acta **950**:21–29.
40. **Yang, L., T. C. Rowe, E. M. Nelson, and L. F. Liu.** 1985. *In vivo* mapping of DNA topoisomerase II-specific cleavage sites on SV40 chromatin. Cell **41**:127–132.