ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1986, p. 1073–1078
0066-4804/86/061073-06$02.00/0

Vol. 29, No. 6

Effect of 4-Quinolones and Novobiocin on Calf Thymus DNA Polymerase α Primase Complex, Topoisomerases I and II, and Growth of Mammalian Lymphoblasts

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Received 25 September 1985/Accepted 26 March 1986

The influence of ciprofloxacin, nalidixic acid, norfloxacin, novobiocin, and ofloxacin on elements of eucaryotic DNA replication was investigated in vitro. Each of the 4-quinolones, when present in amounts of more than 100 μg/ml, reversibly inhibited the DNA synthesis performed by the 9S DNA polymerase α primase complex from calf thymus. Novobiocin at 500 μg/ml or at higher concentrations irreversibly inactivated DNA polymerase α primase complex. The accuracy of in vitro DNA synthesis in the absence of repair mechanisms was determined from amber-revertant assays with φX174am16(+) DNA as template. The antimicrobial agents did not significantly increase the frequencies of base pairing mismatches during the course of replication, indicating that the basal mutation rate is not affected by novobiocin and the 4-quinolones. The K_i values of 50% inhibition of DNA topoisomerases from calf thymus by ciprofloxacin, norfloxacin, novobiocin, nalidixic acid, and ofloxacin were 300, 400, 1,000 or more, 1,000 or more, and 1,500 or more μg/ml, respectively, in the case of topoisomerase I, and the K_i values were 150, 300, 500, 1,000, and 1,300 μg/ml, respectively, in the case of topoisomerase II. The procaryotic topoisomerase II is approximately 100-fold more sensitive to inhibition by ciprofloxacin, norfloxacin, and ofloxacin than is its eucaryotic counterpart. Growth curves of lymphoblasts were recorded in the presence of ofloxacin and ciprofloxacin. Neither 1 nor 10 μg of ciprofloxacin or of ofloxacin per ml affected cell proliferation. Ofloxacin and ciprofloxacin at 100 μg/ml inhibited cell growth; 1,000 μg/ml led to cell death. No correlation exists between the antimicrobial and cytotoxic activities of the 4-quinolones.

The nalidixic acid analogs ciprofloxacin, norfloxacin, and ofloxacin are highly active bactericidal agents, effective against a broad spectrum of gram-positive and gram-negative bacteria (3, 22, 31, 37). The MICs generally range between 0.01 and 10 μg/ml. According to the outcome of the first clinical trials, the new 4-quinolones show promise as safe and effective antimicrobial agents (see Proc. 14th Int. Congr. Chemother., 1985).

The antibacterial activity of the 4-quinolones is probably caused by the inhibition of DNA synthesis (15, 35, 38, 40). The drugs inhibit DNA gyrase, a bacterial type II topoisomerase that negatively supercoils DNA (15, 35). According to Sato et al. (K. Sato, Y. Inoue, S. Yamashita, M. Inoue, and S. Mitsuhashi, Proc. 14th Int. Congr. Chemother., p. 49, 1985), ciprofloxacin, norfloxacin, and ofloxacin inhibit the ATP-dependent supercoiling activity and the ATP-independent relaxing activity of the DNA gyrase from *Escherichia coli* and *Pseudomonas aeruginosa* at concentrations ranging from 0.1 to 10 μg/ml. The subunit A of DNA gyrase is assumed to be the direct target of the 4-quinolones (15, 35); however, recently published data provide evidence that binding to single-stranded regions of DNA may be the primary mechanism of drug action (38). The subunit B of DNA gyrase is inhibited by novobiocin (15, 35).

The DNA gyrases belong to the type II topoisomerases which alter the topology of DNA by passing a DNA helix through a transient double-stranded break in another helical structure, changing the linking number by ±2 (15). In contrast, the type I enzymes are able to relieve torsional constraints in DNA by making a transient single-stranded nick and by changing the linking number by ±1. Besides their effects on bacterial topoisomerase II, both novobiocin and nalidixic acid are known to inhibit eucaryotic topoisomerases purified from HeLa cells (29), rat liver (8), and embryos of *Drosophila melanogaster* (34). The eucaryotic topoisomerase II was slightly more resistant (5-fold or less) to inhibition by nalidixic acid and was about 1,000-fold more resistant to inhibition by novobiocin than was its bacterial counterpart (29, 30, 34).

Nalidixic acid and novobiocin not only inhibit the enzymes modifying DNA topology but also interfere with the other elements of procaryotic and eucaryotic DNA replication. Nalidixic acid, e.g., alters the chainlength distribution of replication products synthesized by the murine DNA polymerase (6). Inhibition of DNA polymerase from other mammals by novobiocin has been reported (4, 7, 11, 24, 25, 32, 36, 42). Moreover, nalidixic acid and novobiocin influence DNA repair (5, 20, 26, 27). In *E. coli*, nalidixic acid is an effective inducer of SOS functions (20). Studies on human fibroblast strains demonstrated that amounts of novobiocin and nalidixic acid as low as 5 μg/ml may be sufficient to retard repair-type DNA synthesis (26). So far, there are no reports in the literature on whether the new 4-quinolones ciprofloxacin, norfloxacin, and ofloxacin interfere with eucaryotic DNA replication. Hence, we tested the influence of nalidixic acid, novobiocin, and the new 4-quinolones on the function of topoisomerases I and II and on DNA synthesis by the polymerase α primase complex. The fidelity of DNA replication was determined by copying bacteriophage φX174am16 single-stranded DNA with polymerase α in vitro (19). As an overall estimate of the influence of the 4-quinolones on replicative DNA synthesis, we measured the proliferation of cells of human lymphoblastoid lines which

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were tested previously for susceptibility to DNA-damaging drugs (21).

MATERIALS AND METHODS

Chemicals. Nucleotides were purchased from Boehringer Mannheim Biochemicals, [α-^{32}P]dATP was purchased from New England Nuclear Corp., ethidium bromide, protamine, and spermine were purchased from Serva, bovine serum albumin (BSA) was purchased from Biotest Frankfurt, and agarose was purchased from Bethesda Research Laboratories, Inc. Nalidixic acid (Sigma Chemical Co.), novobiocin (Sigma), norfloxacin (Merck Sharp & Dohme), ofloxacin (Hoechst), and ciprofloxacin (Bayer) were used as obtained from the manufacturers without further purification. Stock solutions of antibiotics contained 10% (vol/vol) dimethyl sulfoxide (DMSO). All other chemicals were of at least analytical grade.

Bacterial strains and media. *E. coli* CQ_2 (F^- *supF*), C (F^-, wild type) (39), C600 (F^- *thr leu thi lacY tonA supE*) (1), and JM103 [Δ(*lac-pro*) *supE thi strA sbcB15 hsd124* F *traD36 proAB lacI^{q}ZM13*] (28) were grown in LB broth. Soft agar contained in addition 0.72 g of Bacto-Agar (Difco Laboratories) per liter and 15 g of L agar per liter. LTAS soft agar was supplemented with 10% sucrose, 1% (vol/vol) BSA, and 10 mM $CaCl_2$. Strain CQ_2 was host for phage ϕX174am16 (12, 13), and strain JM103 was host for phage M13mp7.

DNA substrates. Activated DNA as template primer was obtained by the digestion of 100 mg of calf thymus DNA in 40 ml of a 5 mM $MgCl_2$–10 mM Tris buffer (pH 7.8) mixture with 125 μg of DNase for 15 min at 37°C. The reaction was stopped by subsequent incubation at 77°C for 5 min and then at 65°C for 10 min (2). Single-stranded ϕX174am16(+) and M13mp7(+) DNAs were prepared from polyethylene glycol precipitates by the method of Yamamoto et al. (47). The double-stranded supercoiled replicative form I (RFI), of ϕX174am16 was isolated from 6- to 8-h fermentations of phage-infected *E. coli* CQ_2 cells in the presence of chloramphenicol (17). The DNA substrates were more than 99% pure as checked by agarose gel electrophoresis.

DNA synthesis assays. Calf thymus DNA polymerase α primase complex (9S enzyme) was prepared to a specific activity of 50,000 U/mg as described previously (18). One unit is defined as the amount of enzyme which catalyzes the incorporation of 1 nmol of dTMP per h at 37°C with activated DNA as template primer. The DNA synthesis assays each (50 μl) contained 20 mM Tris acetate (pH 7.2), 75 mM potassium acetate, 5 mM dithioerythritol, 0.1 mg of BSA per ml, 0.1 mM deoxynucleoside triphosphate, and various concentrations of antimicrobial agents. When activated DNA was used, the initial rate of DNA synthesis was measured by incubation of the assay mixture for 10 min at 37°C in the presence of 0.5 mg of DNA per ml and 100 U of DNA polymerase α primase complex per ml. For the replication of single-stranded phage DNA, each assay mixture was supplemented with ribonucleotides (1 mM ATP, 0.1 mM CTP, 0.1 mM GTP, and 0.1 mM UTP) as substrates for the primase activity of DNA polymerase α. Replication of M13mp7(+) (10 μg/ml) was performed with 100 U of DNA polymerase α per ml (90 min, 37°C); replication of ϕX174am16(+) (20 μg/ml) was performed with 300 U of enzyme per ml (90 min, 37°C). The incorporation of nucleotides was followed by inclusion of [α-^{32}P]dATP into the assay mixture. After incubation, a portion was pipetted onto a Whatman GF/C filter disk. The filter disk was incubated for 10 min in ice-cold trichloroacetic acid (10% [vol/vol], 10 mM $Na_4P_2O_7$), washed with 20 ml of 1 N HCl and 5 ml of ethanol, and dried. The acid-precipitated radioactivity was determined by liquid scintillation counting.

Amber-revertant assays. The principle of the amber-revertant assay has been described previously (12, 45). To determine the basal mutation rate in the absence of repair mechanisms, the DNA of the amber mutant am16 of the bacteriophage ϕX174 is copied by the DNA polymerase α primase complex in vitro, and the DNA is expressed in vivo to produce progeny. The accuracy of DNA replication is measured by scoring the proportion of revertants to amber stock in the progeny.

Preparation of spheroplasts. A 100-ml amount of L broth supplemented with 0.4 mg of vitamin B_1 was inoculated with 2.5 ml of an overnight culture of *E. coli* C600. Cells were grown in an orbital shaker at 37°C and were harvested in the logarithmic phase by centrifugation (10 min, 3,000 $\times$ *g*). The pellet was suspended in 0.5 ml of 30% (vol/vol) BSA and 1 ml of 1.5 M sucrose. After the addition of 50 μl of lysozyme (2 mg/ml in 250 mM Tris hydrochloride [pH 7.8]) and 200 μl of 4% (vol/vol) EDTA, the suspension was incubated for 2 min at room temperature. Subsequently, 17.8 ml of PA medium, made up of 10% sucrose, 0.1% glucose, 0.05% (vol/vol) Casamino Acids (Difco), was added slowly. After another 12 min, 0.3 ml of 10% $MgSO_4$, 37.5 μl of 1% (vol/vol) protamine sulfate, and 50 μl of spermine chloride (250 mg/ml) were added, and the spheroplast preparation was put on ice. After 3 h, the spheroplasts were competent for the uptake of phage DNA.

Replication and transfection. ϕX174am16(+) DNA was replicated in a 50-μl reaction mixture for 90 min at 37°C by DNA polymerase α in the presence of novobiocin or 4-quinolones as described above. DNA was precipitated by the addition of 2.5 volumes of ethanol and 0.1 volume of 3 M ammonium acetate. After 20 min of incubation at −80°C, the sample was centrifuged for 15 min at 4°C in an Eppendorf centrifuge. The pellet was lyophilized and dissolved in 50 μl of 10 mM Tris hydrochloride [pH 7.8]–1 mM EDTA. A portion of 10 μl, in the case of the progeny-revertant assay, and 10 μl of a 10^3- to 10^4-fold dilution, in the case of the progeny-expression assay, were mixed with 0.4 ml of 50 mM Tris hydrochloride [pH 8.1]–1 mM EDTA and then with 0.4 ml of the spheroplast suspension. After 10 min of incubation at 30°C, the spheroplasts were mixed with 2.5 ml of prewarmed LTAS soft agar (45°C) and 250 μl of a suspension of indicator bacteria in logarithmic phase. The mixture was plated immediately on L agar. The *supF* strain of *E. coli* CQ_2 was used for the expression of unchanged progenies, and the wild-type strain of *E. coli* C was used for the expression of revertants. Plaques were scored for *E. coli* CQ_2 plates after 4 h of incubation at 37°C, and plaques were scored for *E. coli* after 8 h of incubation at 30°C.

Assays of DNA topoisomerases I and II. The purification procedures for DNA topoisomerases I and II from calf thymus will be published separately. The enzyme preparations were free of proteases, nucleases, and DNA polymerases. Topoisomerase II was purified to near homogeneity and was not contaminated by topoisomerase I activity.

The activity of topoisomerase I in the presence and absence of 4-quinolones or novobiocin was assayed by measurement of the relaxation of supercoiled double-stranded DNA in the absence of ATP and Mg^{2+} ions. The reaction mixture (20 μl), containing 20 mM Tris hydrochloride (pH 7.6), 5 mM EDTA, 0.2 M KCl, 50 μg of BSA per ml, 50 μg of superhelical ϕX174am16 per ml, and 100 to 250 U of topoisomerase I, was incubated with the antimicrobial

agents for 30 min at 37°C. The reaction mixture contained 1 μg of protein per ml of topoisomerase I preparation with a specific activity of 2×10^7 U/mg. One unit was defined as the amount of enzyme required to achieve complete relaxation of 0.5 μg of supercoiled φX174 DNA in 15 min at 37°C. The products were separated by agarose gel electrophoresis as described in the next paragraph.

Topoisomerase II activity was assayed by the catenation of double-stranded supercoiled DNA in the presence of histone H1, ATP, and Mg^{2+} ions. The catenation can only be observed in a narrow concentration range of histone H1, and hence the optimum amount of H1 in the assay was determined separately for each enzyme preparation and enzyme concentration. The reaction was performed in a 20-μl volume containing 100 mM Tris hydrochloride (pH 7.4), 0.1 mM EDTA, 5 mM $MgCl_2$, 50 mM NaCl, 50 mM KCl, 1.5 mM β-mercaptoethanol, 2 mM ATP, 30 μg of BSA per ml, 50 μg of supercoiled double-stranded DNA (φX174am16 RF1) per ml, about 8 to 12 μg of histone H1 per ml, 5 U of homogeneous topoisomerase II preparations, and various concentrations of antimicrobial agents dissolved in 10% (vol/vol) DMSO. The sample was incubated for 30 min at 37°C, and the products were separated on a neutral agarose gel. One unit of topoisomerase II activity was defined as the amount of enzyme required to achieve complete catenation of 0.5 μg of supercoiled φX174 DNA in 1 h at 37°C. The specific activities of the topoisomerase II preparations varied between 5×10^5 and 2×10^6 U/mg of protein, which leads to an enzyme concentration of approximately 0.7 nM in the assay. Under the chosen experimental conditions, topoisomerase II catalyzed both the catenation and the relaxation of supercoiled DNA, and, accordingly, the two products of catenated and relaxed rings were always observed. The homogeneous topoisomerase II preparations did not contain any nicking activity.

For both topoisomerases I and II, the 50% inhibition of relaxation and catenation was determined visually from repetitive titrations narrowing the concentration range of drug from titration to titration.

Agarose gel electrophoresis. Neutral agarose gels were prepared as horizontal slabs from a solution of 1% agarose in 90 mM Tris phosphate (pH 8.3)–5 mM sodium acetate–2 mM EDTA. The loading buffer contained 250 mM EDTA (pH 8.2), 0.2% sodium dodecyl sulfate, 0.1% bromphenol blue, and 25% sucrose. Samples were prepared for electrophoresis by the addition of 5 μl of loading buffer to 10 μl of the respective reaction mixture. Electrophoresis was performed at 80 mA for 1 to 3 h. The gel was stained with ethidium bromide (5 μg/ml), and the DNA was visualized with a UV lamp at 256 nm.

Lymphoblastoid cell lines and cell culture. The lymphoblast cell lines HSC49, HSC50, HSC89, HSC92, and HSC93 were derived from peripheral blood lymphocytes by the method of Glade and Broder (16). Cells were grown in alpha medium (41) supplemented with 15% fetal calf serum without antibiotics. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, and the culture medium was changed twice a week.

Growth inhibition experiments. Lymphoblasts (1.5×10^4) were seeded in 0.3-ml aliquots into each well of a 96-well plate (Becton Dickinson Labware). Cells were grown in the absence or presence of various concentrations of ofloxacin or ciprofloxacin. Cell viability, defined by the exclusion of a 0.4% (wt/vol) aqueous trypan blue solution, and cell number were determined at 24-h intervals in a hemacytometer over a period of 7 days. The sensitivity of a cell line was defined as the drug concentration yielding a 50% inhibition of growth (EC_{50}). The growth percentage (EC value) was defined as follows: EC = 100 × (number of drug-treated cells on the final day of the growth curve − initial cell number)/(number of cells in the control well on the final day − initial cell number).

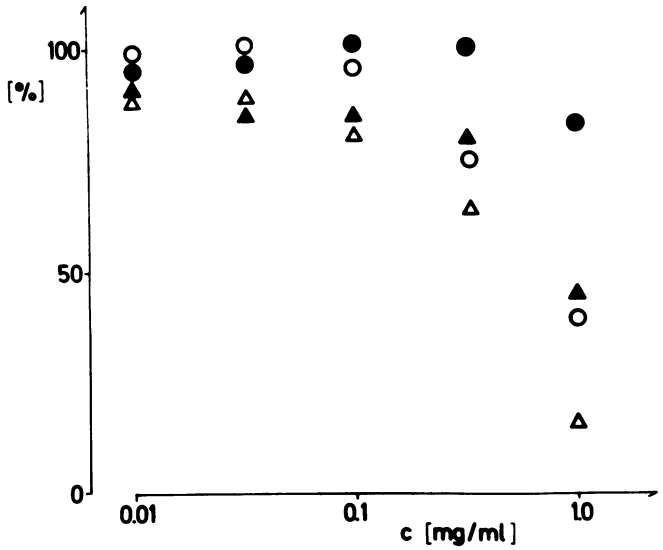

FIG. 1. Enzymatic activity of DNA polymerase α primase complex in the presence of 4-quinolones in percent activity of controls without antimicrobial agents. ○, Norfloxacin; ●, ofloxacin; △, ciprofloxacin; ▲, nalidixic acid. The size of the symbols indicates the mean ± the standard error of the mean.

RESULTS

DNA synthesis assays. With activated DNA as template primer, the DNA synthesis by the 9S DNA polymerase α primase complex from calf thymus was measured in the presence of 0, 10, 33, 100, 333, and 1,000 μg of 4-quinolones per ml. A significant decrease in the amount of synthesized DNA was only observed with drug concentrations of more than 100 μg/ml (Fig. 1). An amount of 1 mg/ml significantly reduced the activity of DNA polymerase α by about 20% in the case of ofloxacin, by approximately 60% in the case of nalidixic acid and norfloxacin, and by more than 80% in the case of ciprofloxacin. This inhibition of DNA polymerase α primase complex was reversible. Dilution of the reaction mixture with buffer restored the initial enzyme activity. Control experiments with various amounts of DMSO revealed that 10% (vol/vol) DMSO in the assay had no influence on DNA synthesis and the accuracy of DNA replication.

In agreement with data from the literature (11, 42), novobiocin was found to be an effective inhibitor of DNA polymerase α primase complex. Taking a 100-μg/ml concentration of activated DNA as template, the enzymatic activity linearly decreased from 100% to nearly zero in the narrow concentration range from 0.4 to 0.8 mg of novobiocin per ml. Concentrations of activated DNA of more than 10 μg/ml reduced the enzymatic activity by about 20% per added 100 μg/ml (substrate inhibition). Assays with M13mp7(+) DNA as a template of definite length and sequence yielded the same result. Measurements of the Michaelis-Menten kinetics of DNA synthesis as a function of deoxynucleotide concentration revealed that with increasing novobiocin concentration the K_m and V_{max} values decreased. Thus, novobiocin did

TABLE 1. Amber-revertant assays: error rates of the DNA polymerase α primase complex in the presence of antimicrobial agent at 300 μg/ml

Antimicrobial agent	Error rate of DNA polymerase α primase complex[a]
Ciprofloxacin	$(4 \pm 2)\ 10^{-5}$
Nalidixic acid	$(6 \pm 4)\ 10^{-5}$
Novobiocin	$(8 \pm 4)\ 10^{-5}$
Ofloxacin	$(8 \pm 3)\ 10^{-5}$
Control	$(3 \pm 2)\ 10^{-5}$
Control + 1 mM Mn^{2+}	$(3 \pm 1)\ 10^{-4}$

[a] Values are the mean ± standard error of the mean from at least four separate experiments. For each experiment, bacteria were plated in triplicate. All the values are corrected for mismatch repair and minus-strand expression by a factor of 0.38 (19). Replication of single-stranded M13mp7 DNA in the presence of Mn^{2+} was initiated with the *Hae*III fragment H15A of φX174 (19).

not act as a reversible competitive or noncompetitive inhibitor. When the reaction mixture was preincubated for 10 min with the completely inhibiting novobiocin concentration of 1 mg/ml and subsequently diluted with buffer to less-inhibiting concentrations of 0.5 or 0.25 mg/ml, the enzymatic activity was not restored. We conclude that novobiocin irreversibly inactivates mammalian DNA polymerase α primase complex.

Accuracy of DNA polymerase α primase complex in the presence of the antimicrobial agents. The error rates of DNA replication were analyzed by amber-revertant assays. The 90-min incubation with DNA polymerase α primase complex at 37°C yielded mainly the complete double-stranded relaxed circle of φX174 (RFII) and some linear double-stranded RFIII as by-product. In some assays, nalidixic acid led to overreplication, indicating strand-displacement synthesis.

The misinsertion frequency at the amber codon was determined to be in the range of 10^{-4} to 10^{-5}. The presence of 4-quinolones or novobiocin had no significant influence on the mean error rate of the DNA polymerase α primase complex (Table 1). As a positive control of the assay, the obligatory cation Mg^{2+} was substituted by 1 mM mutagenic cation Mn^{2+}. In the presence of manganese ions, a 10-fold decrease of accuracy was observed. This result is in accordance with data obtained by Kunkel and Loeb (23) with the φX174am3-revertant assay and DNA polymerase I.

Inhibition of eucaryotic DNA topoisomerases I and II by novobiocin and 4-quinolones. Novobiocin, nalidixic acid, and ofloxacin had no effect on the eucaryotic topoisomerase I from calf thymus in the concentration range of 1 to 1,000 μg/ml. On the other hand, norfloxacin and ciprofloxacin were found to be inhibitors of topoisomerase I. The K_i values of 50% inhibition by these drugs were determined to be between 300 and 400 μg/ml (Table 2). Norfloxacin or ciprofloxacin at 100 μg/ml was sufficient to resolve the various intermediate topoisomers of the incomplete relaxation of supercoiled DNA in the agarose gel (Fig. 2).

Topoisomerase II from calf thymus was more strongly affected by the antibiotics than was topoisomerase I. The K_i values of 50% inhibition varied within one order of magnitude, between 150 and 1,300 μg/ml for the various drugs (Table 2). Amounts of less than 50 μg/ml were found to be noninhibitory for topoisomerase II. The variation of the pH of the stock solution of the antimicrobial agent between 6 and 8 had no influence on the results. The potency of inhibition of topoisomerase II increased in the order ofloxacin < nalidixic acid < novobiocin < norfloxacin < ciprofloxacin. Ofloxacin and nalidixic acid were found to be weak inhibitors. Only high concentrations of antibiotic, more than 1 mg/ml, induced the occurrence of intermediate topoisomers. A 10% concentration of DMSO (vol/vol) in the assays did not influence the activity of topoisomerases I and II.

Growth curves of lymphoblasts in the presence of ofloxacin and ciprofloxacin. The growth of the cell lines was defined by cell number and viability. Samples were taken during the stage of the onset of growth, during the exponential growth phase, and in the late-logarithmic phase. Low amounts of ofloxacin, 1 or 10 μg/ml, delayed the onset of growth in vitro by 1 to 2 days without affecting cell viability. Ciprofloxacin at 1 and 10 μg/ml had no effect on cell proliferation when compared with controls. Ofloxacin at 100 μg/ml slightly inhibited cell growth by about 20% (statistically not significant). Ciprofloxacin at 100 μg/ml inhibited cell growth completely. Ofloxacin and ciprofloxacin at 1 mg/ml induced the cell death of lymphoblasts.

DISCUSSION

The purpose of this study was to discover whether the recently developed nalidixic acid analogs interfere with elements of eucaryotic DNA replication. Our data demonstrate that the 4-quinolone antimicrobial agents are able to inhibit mammalian DNA polymerase α primase complex and DNA topoisomerases I and II. However, amounts of at least 50 μg of drug per ml were needed before an inhibitory effect on the function of the eucaryotic enzymes could be observed.

Interestingly, no correlation exists between the antimicrobial activity of a drug and its cytotoxic ability to inhibit eucaryotic DNA replication. Ciprofloxacin and ofloxacin are the most active antibacterial agents within the series of drugs investigated. Our in vitro assays revealed that ciprofloxacin is the most potent inhibitor of the topoisomerases and polymerase α and that ofloxacin is the weakest inhibitor of these mammalian enzymes.

TABLE 2. K_i values of 50% inhibition (K_{i50}) of DNA topoisomerases by novobiocin and 4-quinolones

Antimicrobial agent	K_{i50} (μg/ml) of inhibition of[a]:					
	Topoisomerase I from calf thymus	Topoisomerase II from:			Gyrase from:	
		Calf thymus	HeLa cells (29)	*D. melanogaster* (34)	*E. coli* KL-16	*P. aeruginosa* PAO1
Ciprofloxacin	300	150			1.0[b]	1.6[b]
Nalidixic acid	>1,000	1,000	500	600	200 (30)	600 (30)
Norfloxacin	400	300			2.4[b]	4.4[b]
Novobiocin	>1,000	500	200	130	0.1 (30)	0.1 (30)
Ofloxacin	>1,500	1,300			3.1[b]	6.4[b]

[a] The relative accuracy of the K_i values is approximately 30%. The references from which data were taken are given in parentheses.
[b] K. Sato, Y. Inoue, S. Yamashita, M. Inoue, and S. Matsuhashi, Proc. 14th Int. Congr. Chemother., 1985, p. 49.

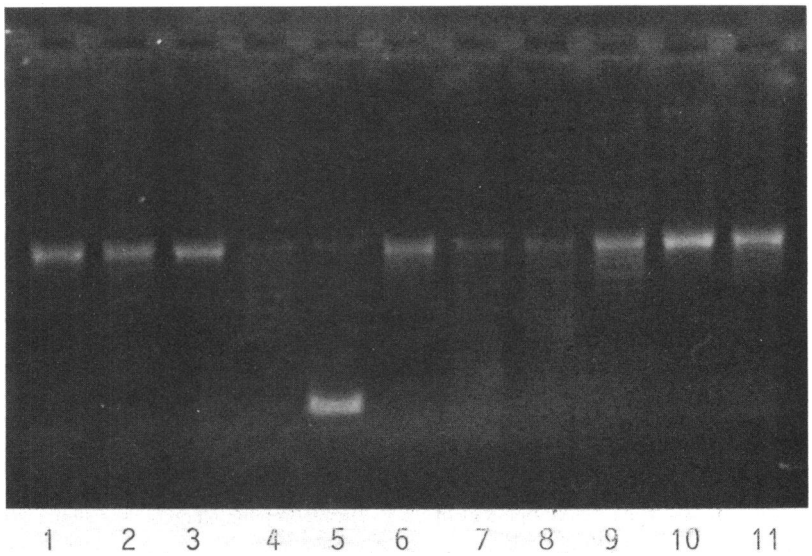

FIG. 2. Relaxation of supercoiled double-stranded φX174am16 DNA (RFI) by topoisomerase I in the presence of novobiocin or 4-quinolones. Lanes: 1, novobiocin (1 mg/ml); 2, nalidixic acid (1 mg/ml); 3, ofloxacin (1 mg/ml); 4, norfloxacin (1 mg/ml); 5, control (RFI and RFII of φX174am16); 6, control (no antimicrobial agent); 7, ciprofloxacin (1 mg/ml); 8, ciprofloxacin (0.67 mg/ml); 9, ciprofloxacin (0.33 mg/ml); 10, ciprofloxacin (0.1 mg/ml); 11, ciprofloxacin (0.01 mg/ml).

The 4-quinolones only slightly affected DNA synthesis by DNA polymerase α primase complex from calf thymus. Even in the presence of 1 mg of drug per ml, DNA synthesis was only reduced by 20 to 80%. The ability of nalidixic acid to inhibit DNA polymerase α primase complex is comparable to its ability to inhibit eucaryotic aminoacyl-tRNA synthetases (46).

The amber-revertant assays revealed that novobiocin and the 4-quinolones do not impair the accuracy of DNA polymerase α primase complex. Because the test measures the basal mutation rates of eucaryotic DNA replication in vitro in the absence of repair mechanisms, it may represent a useful supplement to the common tests of mutagenicity. The only practical disadvantage is its requirement of a pure DNA polymerase preparation.

DNA topoisomerases are essential for the function of eucaryotic cells (9, 10, 14, 33, 43, 44, 48). The inhibition of these enzymes would be an undesirable side effect of antimicrobial therapy with 4-quinolones. However, the K_i values are relatively high. The minimum concentration to inhibit topoisomerase II in vitro was determined to be about 50 μg/ml. Table 2 presents the K_i values of 50% inhibition of procaryotic and eucaryotic DNA topoisomerase II by the 4-quinolones. The selectivity of nalidixic acid between the bacterial enzyme and its eucaryotic counterpart is poor; the K_i values are within the same order of magnitude. However, in the cases of ciprofloxacin, ofloxacin, and norfloxacin, the procaryotic type II topoisomerase is about two orders of magnitude more sensitive to inhibition by the 4-quinolones than is the eucaryotic enzyme.

The cell growth of lymphoblasts was inhibited 50% by ciprofloxacin concentrations of approximately 100 μM or ofloxacin concentrations of 300 μM. These EC_{50} values are high compared with DNA-modifying drugs which are known to be cytotoxic for eucaryotic cells (Table 3). The same lymphoblast cell lines were about 10-fold more sensitive to the nitrosourea compound 1,3-bis(2-chloroethyl)-*N*-nitrosourea and about 100- to 1,000-fold more sensitive to DNA-cross-linking agents (*cis*-platinum, mitomycin C, melphalan) and to the monofunctional alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (21). Our EC_{50} values of ofloxacin and ciprofloxacin for lymphoblasts are comparable to the data reported by M. Negishi, C. Young, and H. Suzuki (Proc. 14th Int. Congr. Chemother., p. 129, 1985) for the inhibition of C57BL mouse bone marrow cells as hemopoietic stem cells by ofloxacin, norfloxacin, and nalidixic acid.

TABLE 3. Sensitivity of lymphoblasts to ciprofloxacin, ofloxacin, and various DNA-damaging chemicals[a]

Compound	EC_{50} (μM)
Ciprofloxacin	≈100
Ofloxacin	≈300
BCNU[b]	13 ± 4
MNNG[c]	1.1 ± 0.6
cis-Platinum[d]	0.6 ± 0.1
Melphalan	1.0 ± 0.4
Mitomycin C	0.08 ± 0.03

[a] The standard deviation of the EC_{50} values of ciprofloxacin and ofloxacin was determined to be about 30%. The values were determined from growth curves performed in triplicate with five cell lines. The EC_{50} values of the DNA-damaging agents are taken from reference 21. Mean and standard deviation were evaluated from two to four experiments.

[b] BCNU, 1,3-bis(2-Chloroethyl)-*N*-nitrosourea.

[c] MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

[d] *cis*-Platinum, *cis*-Diaminedichloroplatinum (II).

ACKNOWLEDGMENTS

E. coli JM103 and the phage M13mp7 were gifts from Bethesda Research Laboratories, Inc. *E. coli* C, CQ_2, and C600 were supplied by A. R. Fersht, Imperial College, London. The lymphoblast strains were donated by J. R. Riordan and M. Buchwald, Research Institute, The Hospital for Sick Children, Toronto, Canada. We thank M. Wehsling for expert technical assistance.

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