Journal of Cellular Biochemistry 51:165–174 (1993)

4-Quinolones Cause a Selective Loss of Mitochondrial DNA From Mouse L1210 Leukemia Cells

Jeffrey W. Lawrence, Sandra Darkin-Rattray, Fan Xie, Allen H. Neims, and Thomas C. Rowe

Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32610-0267

Abstract The 4-quinolone antibiotics nalidixic acid and ciprofloxacin are potent inhibitors of the bacterial type II topoisomerase DNA gyrase. Treatment of mouse L1210 leukemia cells with these drugs resulted in a delayed inhibition of cell proliferation. Prior to inhibition of cell proliferation, there was a time-dependent decrease in the cellular content of mitochondrial DNA (mtDNA). The decrease in mtDNA was associated with a decrease in the rate of mitochondrial respiration and an increase in the concentration of lactate in the growth medium. Inhibition of cell proliferation by 4-quinolones was reversible upon drug washout. However, there was a 2- to 4-day lag before the growth rate returned to normal levels. This was preceeded by an increase in mtDNA content and mitochondrial respiration. These studies suggest that inhibition of mammalian cell proliferation by 4-quinolone drugs is related to the selective depletion of mtDNA.

Key words: cell growth inhibitors, nalidixic acid, ciprofloxacin, DNA gyrase, topoisomerase

The 4-quinolones are a class of antibacterial drugs that act by inhibiting the type II topoisomerase DNA gyrase [Drlica and Franco, 1988; Gootz et al., 1990]. The structures of the 4-quinolones, nalidixic acid and ciprofloxacin, are shown in Figure 1. At very high concentrations (approximately 100-fold higher than those required to inhibit bacterial growth), these agents inhibit the growth of mammalian cells [Hussy et al., 1986]. There is some evidence that the inhibition of mammalian cell growth may be related to effects of these drugs on mtDNA synthesis. Castora et al. [1983] have shown that replication of mtDNA in isolated rat mitochondria is inhibited in the presence of the 4-quinolone drugs, nalidixic acid and oxolinic acid. These investigators suggested that these drugs might be acting by inhibiting a mitochondrial DNA gyrase-like enzyme that functioned to facilitate changes in the topology of mtDNA that were required for replication. This is consistent with recent studies in the trypanosomatid *Crithidia fasiculata* that have identified a mitochondrial type II topoisomerase that is inhibited by the 4-quinolone, nalidixic acid [Melendy and Ray, 1989]. These studies have led us to investigate whether the inhibition of mammalian cell growth by 4-quinolones is related to alterations in the content of mtDNA using mouse leukemia L1210 cells as a model.

Abbreviations used: MEM, minimal essential media; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline (10 mM $NaHPO_4$ (pH 7.4), 137 mM NaCl, 2.5 mM KCl, 1.5 mM KH_2PO_4); ddC, 2',3'-dideoxycytidine; COX, cytochrome c oxidase; VM-26, 4'-demethylepipodophyllotoxin-9-(4,6-O-thenylidene-B-D-glucopyrano side); FACS, fluorescence activated cell sorter.

Received August 21, 1992; accepted August 26, 1992.

Address reprint requests to Thomas C. Rowe, Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Box 100267, J.H. Miller Health Center, Gainesville, FL 32610-0267.

MATERIALS AND METHODS

Materials

Agarose and RNase T1 were obtained from Bethesda Research Laboratories (Gaithersburg, MD). Nalidixic acid was purchased from Sigma (St. Louis, MO). Ciprofloxacin was kindly provided from Miles Pharmaceuticals (West Haven, CT). Proteinase K was from Boehringer Mannheim Biochemicals (Indianapolis, IN) and nitrocellulose BA85 was from Schleicher and Schuell (Keene, NH).

Cell Culture

Mouse leukemia L1210 cells, a cell line with a doubling time of approximately 12 h, were obtained from Dr. William Hauswirth (University

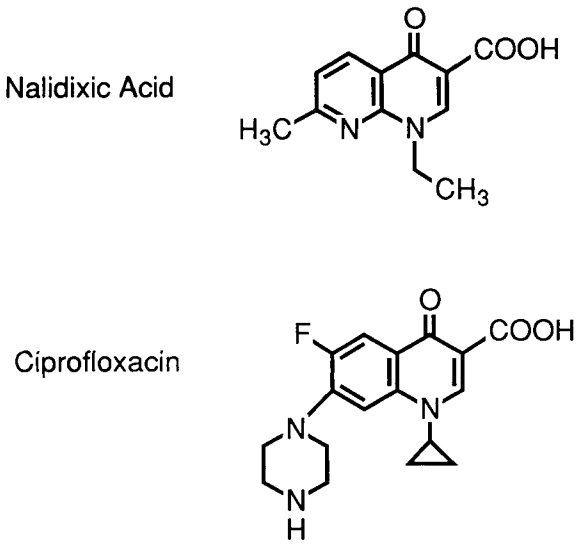

Fig. 1. Structures of nalidixic acid and ciprofloxacin.

of Florida). Cells were maintained as a suspension in α-MEM[1] media supplemented with 10% fetal bovine serum at 37°C in a 5% CO_2 atmosphere. Cell counts were done with a model Zf Coulter Counter (Hialeah, FL). All cell culture reagents were obtained from GIBCO (Grand Island, NY).

DNA Clones

The human *alu* nuclear repetitive DNA clone Blur 8 [Deininger et al., 1981] was obtained from Dr. L. Liu (Johns Hopkins University). The mouse *per* nuclear repetitive DNA clone M2.5 RI [Shin et al., 1985] was obtained from Dr. T. Bargiello (Rockefeller University). The entire mouse mitochondrial genome, inserted into the *Sac I* site of pSP64, was obtained from Dr. W. Hauswirth at the University of Florida [Hauswirth et al., 1987]. The mouse mitochondrial subclone pMBS-2.1 contains the 2.1-kb *Bam H1–Sac 1* fragment of mouse mitochondrial DNA [Bibb et al., 1981] inserted into the *BamHI–Sac I* site of pSP64. The DNA clones were radiolabeled with [α-^{32}P]dCTP (3,000 Ci/mmol, ICN, Costa Mesa, CA), using a nick-translation kit obtained from Bethesda Research Laboratories (Gaithersburg, MD).

Isolation of Cellular DNA

Total cellular DNA was isolated as described previously [Kroeger and Rowe, 1989]. Briefly, cells were collected by centrifugation at 1,000*g* for 2 min at room temperature. The supernatants were discarded and the cell pellets resuspended in 400 μl of lysis buffer [20 mM Tris–Cl (pH8), 20 mM EDTA, 1% SDS, containing 400 μg proteinase K/ml] and incubated overnight at 37°C. The lysates were then phenol and ether extracted. The residual ether in the samples was removed under vacuum in a Savant Speed-Vac centrifuge then the samples were digested with RNase T1 (6 units) for 2 h at 37°C. Samples were again phenol and ether extracted, the DNA precipitated with 2.5 vol of ethanol, and the resulting precipitates resuspended in 200 μl of TE [10 mM Tris–HCl (pH 8), 1 mM EDTA]. The concentration of DNA was then determined fluorometrically [Labarca and Paigen, 1980], and equal amounts of DNA (10 μg) from each sample were analyzed by Southern hybridization ([Southern, 1975]; see below).

Gel Electrophoresis, Southern Hybridization, and Autoradiography

DNA samples (10 μg in 50 μl TE) were combined with 5 μl of loading buffer (50% sucrose, 0.05% bromophenol blue) and loaded onto a 0.7% agarose horizontal gel in TBE [90 mM Tris–borate (pH 8.3), 2 mM EDTA]. Following electrophoresis at 70 V for 50 h, the gel was stained with ethidium bromide and the DNA visualized using a UV box. The DNA was then transferred onto a nitrocellulose filter and hybridized to nick-translated [^{32}P]-labeled mitochondrial-specific DNA probes as described by Sambrook et al. [1989]. The radioactive mitochondrial DNA signal was directly quantitated from the nitrocellulose filter using a Betascope 603 Blot Analyzer (Betagen Corporation, Waltham, MA); the filter was then autoradiographed at −70°C, using Kodak XAR 5 film and a DuPont Lightning-plus intensifying screen. Variations in the total amount of DNA loaded in each sample were corrected for by removing the mitochondrial DNA probe and then rehybridizing the filter to nuclear-specific *per* or *alu* DNA probes [Shin et al., 1985; Deininger et al., 1981]. The amount of total cellular DNA in each sample was also independently determined from densitometric tracings of the photographic negatives of the ethidium bromide-stained gels.

FACS Analysis

Mouse L1210 cells (1×10^6) were pelleted at 1,000*g* for 2 min and washed with 1 ml PBS before resuspending in 500 μl of PBS. Cells were then incubated on ice for 15 min before adding 500 μl ice cold ethanol. The ethanol was mixed

by gently bubbling air through the sample to minimize cell breakage. After an additional 15 min on ice, cells were pelleted at 1,000*g* for 5 min at room temperature. Cell pellets were then washed once with 1 ml PBS and resuspended in 125 μl RNase buffer (500 U/ml RNase T1, 1.12% sodium citrate). After 10 min at 37°C, cells were stained by the addition of 125 μl propidium iodide solution (50 μg/ml in 1.12% sodium citrate). After staining for 30 min at room temperature, samples were pushed through a 44-μM nylon filter. FACS analysis of the resulting triplicate samples was done with a FACSCAN flow cytometer (Becton-Dickinson, San Jose, CA) at the University of Florida Flow Cytometry Core Laboratory. The data were collected using the CellFIT modeling program and the average fluorescence per cell determined using the LYSYS II program (Becton-Dickson).

Measurement of Mitochondrial Function

Mitochondrial respiration was measured for duplicate samples, using a Clarke-type oxygen electrode as described previously [Esnault et al., 1990]. Briefly, cells (1×10^6) were harvested by centrifugation and resuspended in 30 μl of GMC buffer (8 mM Tris–HCl (pH 7.2), 1 mM potassium phosphate, 14 mM KCl, 1 mM glutamate, 1 mM malate, 30 mM sucrose). A 10-μl aliquot of the cell suspension was then pipeted into a microchamber containing 0.6 ml freshly oxygenated GMC buffer. Polarographic measurements of oxygen consumption were made using a YSI Biological Oxygen Monitor fitted with a Clarke-type oxygen electrode for 10 min at 37°C. Values were expressed as a percent of the non drug-treated control cells.

Cytochrome c oxidase (COX) activity was determined in duplicate, using a spectrophotometric assay [Segal-Bendirdjian et al., 1988]. Briefly, cells (1×10^6) were harvested by centrifugation and washed twice with 0.25 M sucrose before resuspending in 1 ml of H_2O. Cells were then disrupted by 20 strokes in a tight fitting dounce homogenizer. To initiate the assay, 200 μl of the resulting lysate was combined with 100 μl of 0.1 M potassium phosphate (pH 7.0), 70 μl of 1% (w/v) ferrocytochrome c (Sigma type III), and 630 μl H_2O. The reactions were incubated at 37°C and the absorbance at 550 nm measured over a 30-min period against a blank containing 1 ml of 0.01 M potassium phosphate (pH 7.0), 0.07% oxidized ferrocytochrome c (ferrocytochrome c was oxidized by including 1 mM potassium ferricyanide in the blank). The level of activity was expressed relative to the non-drug-treated control cells.

Measurement of Lactate Accumulation

L1210 cells (duplicate samples) were collected by centrifugation and washed and then resuspended in fresh media containing drug. At various times of drug treatment an aliquot of the culture containing 1×10^6 cells was removed and the cells pelleted by centrifugation. The supernatant was then removed and combined with 2 vol of 10% trichloroacetic acid. After 10 min on ice, the sample was clarified by centrifuging at 10,000*g* for 10 min. The clear supernatants were then collected and assayed for lactate content using a kit from Sigma, according to the manufacturer's instructions. The amount of lactate was calculated as mg/10^6 cells and the data expressed relative to the non-drug-treated controls.

RESULTS

Inhibition of Cell Growth by 4-Quinolones

In agreement with earlier studies [Hussy et al., 1986], we found that the 4-quinolone drugs nalidixic acid and ciprofloxacin inhibited the proliferation of mammalian cells. Nalidixic acid inhibited mouse L1210 cell growth at concentrations above 80 μg/ml, with greater than 90% inhibition occurring at 250 μg/ml. Ciprofloxacin was more potent, inhibiting growth at concentrations above 20 μg/ml, with greater than 90% inhibition at 80 μg/ml. An unusual feature of the drug-induced inhibition of growth was that it did not occur until after several days of drug exposure. During the first 2 days of exposure to 250 μg/ml nalidixic acid, there was no apparent slowing in the growth rate (Fig. 2). However by day 4, growth was inhibited by 60%, and by day 10 the cells had essentially stopped dividing. Inhibition of cell growth by nalidixic acid was reversible upon transfer of cells to drug-free media (indicated by an arrow). However, there was a 3- to 4-day lag before growth resumed at normal rates.

Drug-Induced Loss of Mitochondrial DNA

Previous in vitro studies have demonstrated that 4-quinolone drugs inhibit the replication of mitochondrial DNA in isolated mouse mitochondria [Castora et al., 1983]. This led us to investigate whether the delayed inhibition of cell growth

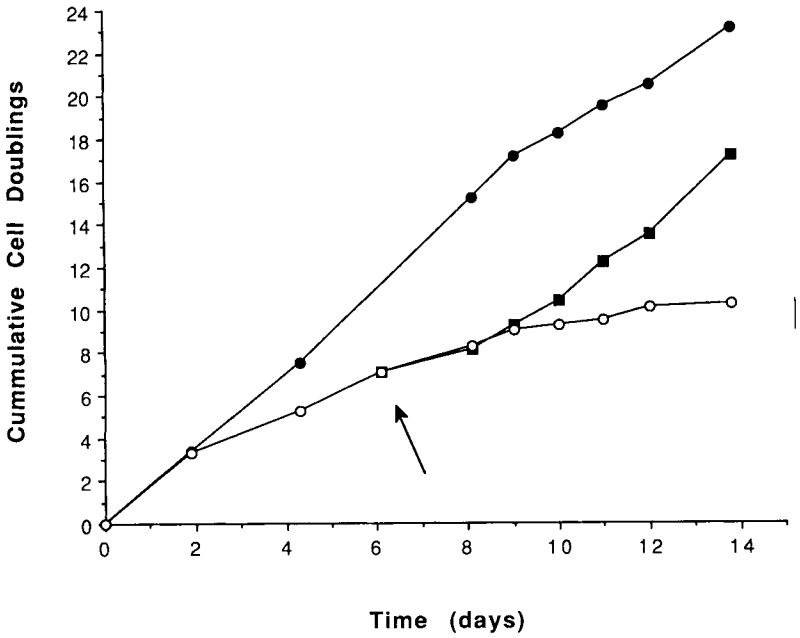

Fig. 2. Effect of nalidixic acid on L1210 cell growth. L1210 cells were grown in T_{25} tissue culture flasks in the presence (○) or absence (●) of 250 μg/ml nalidixic acid. Cell counts were done every 24 h using a Coulter counter to determine the relative increase in cell number. Cells were then spun down and reseeded at a density of 1×10^5 cells/ml in 10 ml fresh media containing drug and the incubation continued. Growth was expressed as the cumulative number of cell doublings. ■, drug-treated cells transferred to drug-free media at day 6 (marked with an arrow).

might be related to a block in mtDNA synthesis. Mammalian mtDNA is a 16- to 17-kb circular duplex molecule encoding 14 tRNA genes, 2 ribosomal RNA subunits, and 13 proteins involved in oxidative phosphorylation [Attardi and Shatz, 1988]. Mammalian cells contain approximately 1,000–2,000 copies of mtDNA representing less than 1% of the total cellular DNA content [Clayton, 1982].

Previous studies have shown that inhibition of mtDNA synthesis by the DNA intercalator ethidium bromide causes a progressive dilution in the cellular content of mtDNA following each round of cell division [Desjardins et al., 1985; Wiseman and Attardi, 1978]. If 4-quinolones inhibit mtDNA synthesis, this should also result in a depletion of the cellular content of mtDNA. Total cellular DNA was isolated from cells exposed to 250 μg/ml nalidixic acid for up to 14 days. Equal amounts of total DNA from each time point were then analyzed for the presence of mtDNA by Southern blotting using a [^{32}P]-labeled mitochondrial DNA probe. As is apparent from the autoradiograph in Figure 3A, nalidixic acid caused a time-dependent loss of mtDNA from mouse L1210 cells. Similar to the inhibition of cell growth, the loss of mtDNA could be reversed by transferring cells to drug-free media. Cells exposed to nalidixic acid for 6 days were washed and then placed in drug-free media for increasing times up to 8 days. Within 4 days there was a significant increase in mtDNA content, and by day 7 the mtDNA content had returned to near control levels. Values for mtDNA content that have been corrected for variations in total cellular DNA are plotted in Figure 3B. These results indicate that mtDNA decreased to 40% of control levels during the first 24 h of drug exposure. The mtDNA content continued to decline, although at a much slower rate, to 20% of control levels by day 14. When drug was removed from the media at day six (marked by an arrow), the mtDNA content returned to near-control levels within 7 days.

Ciprofloxacin was 4- to 5-fold more potent than nalidixic acid in causing a loss of mtDNA from L1210 cells. A dose–response curve showing the effects of ciprofloxacin on mtDNA content is shown in Figure 4. In this experiment, the mtDNA content was measured in cells treated with 20–100 μg/ml ciprofloxacin for 4 days. As is evident from the graph, there was a dose-dependent loss of mtDNA from mouse L1210 cells, with a greater than 80% loss occur-

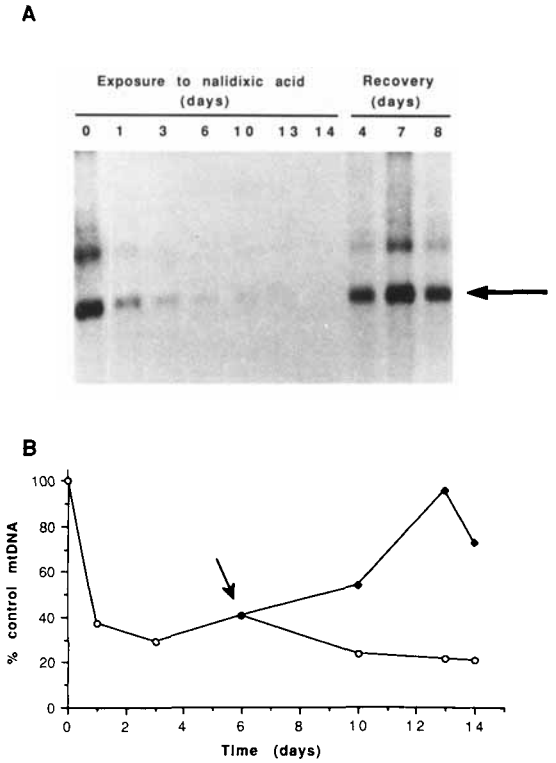

Fig. 3. Effect of nalidixic acid on mouse L1210 cell mtDNA content. L1210 cells were exposed to 250 μg/ml nalidixic acid, and at each time point total cellular DNA was isolated and the mtDNA content was measured as described under Materials and Methods. **A:** Autoradiograph of Southern blot hybridized to a probe containing the entire mouse mitochondrial DNA. **Lane 1,** no drug; **lanes 2–7** contain total DNA from cells continuously treated with 250 μg/ml nalidixic acid for 1, 3, 6, 10, 13, or 14 days; **lanes 8–10** contain total DNA from cells continuously treated with nalidixic acid for 6 days and then placed in drug-free media for 4, 7, or 8 days. The arrow at the right marks the position of supercoiled mtDNA. **B:** Quantitation of the mtDNA content from Southern blot. The radioactive mtDNA signals and corrections for variability in total DNA were quantitated as described under Materials and Methods. ○, cells continuously exposed to nalidixic acid; ●, cells exposed to nalidixic acid for 6 days and then placed in drug-free media. Data are expressed as a percentage of nontreated cells.

ring at ciprofloxacin concentrations above 60 μg/ml.

Although these results suggested that 4-quinolones were inducing a selective depletion of mtDNA from cells, there was a possibility that this apparent decrease was due to an increase in total cellular DNA with no net change in mtDNA content. To investigate this possibility, cells treated with ciprofloxacin were analyzed by flow cytometry to monitor changes in total cellular DNA content (Table I). Cells were continuously exposed to either 80 or 160 μg/ml ciprofloxacin for 1–3 days and then fixed and stained with propidium iodide. The average fluorescence intensity per cell was then determined by flow cytometry to assess changes in total cellular DNA content. There was no significant change in the average cellular DNA content in cells treated for 1 day with ciprofloxacin. Following 3 days of drug exposure, there was still no significant change in the average DNA content per cell except at 160 μg/ml ciprofloxacin. At this concentration there was a 10–20% increase in the average DNA content per cell relative to control samples. These same drug treated samples also showed a similar increase in the percentage of cells in the G2 + M region of the cell cycle. However, the small change in total cellular DNA content observed in cells treated with 160 μg/ml ciprofloxacin still cannot account for the greater than 80% loss of mtDNA.

4-Quinolones Cause a Loss of Mitochondrial Respiration

The drug-induced depletion of mtDNA from mouse L1210 cells led us to examine whether there was a corresponding decrease in mitochondrial respiration. Mitochondrial respiration was monitored by measuring oxygen consumption in cells after treatment with 250 μg/ml nalidixic acid for different lengths of time (Fig. 5A). There was no significant loss of mitochondrial respiration during the first 4 h of drug treatment (data not shown). However, by 24 h, respiration had decreased to 40% of that in control cells. Longer drug exposures of up to 14 days resulted in little additional decrease in respiration. When cells treated with drug for six days were then placed in drug-free media (marked with an arrow), there was a time-dependent recovery of mitochondrial respiration.

The effects of nalidixic acid on mitochondrial COX activity were also measured. This is a multisubunited lipoprotein that forms an important part of the respiratory electron transport chain [Tzagoloff, 1982]. Three of the subunits that make up this protein are encoded for by mtDNA with the remaining subunits being encoded for by nuclear genes [Attardi and Shatz, 1988]. Nalidixic acid caused a decrease in COX activity that paralleled the decrease in mitochondrial respiration. Similarly, there was a time-dependent return of COX activity to control levels when cells were transferred to drug-free culture media.

Ciprofloxacin caused a similar decrease in respiration at concentrations 5 to 10-fold lower

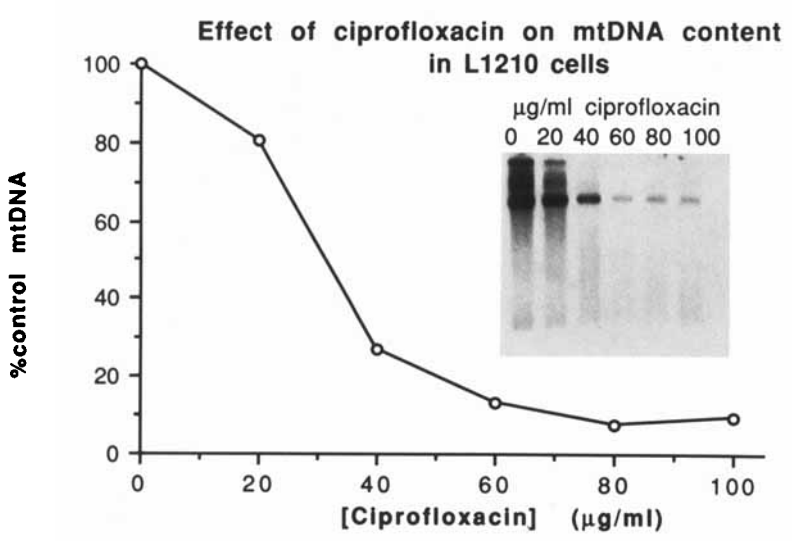

Fig. 4. Effect of ciprofloxacin on mouse L1210 cell mtDNA content. Mouse L1210 cells were exposed to various concentrations of ciprofloxacin and the mtDNA was assessed as in Figure 3. Data are expressed as a percentage of the nontreated cell control. The autoradiograph of the Southern blot that was hybridized with nick-translated mouse mitochondrial probe is shown as an inset. The lanes contain DNA samples from cells treated continuously for 4 days with the indicated concentrations of ciprofloxacin.

TABLE I. FACS Analysis of L1210 Cells Treated With Ciprofloxacin†

Days of exposure (ciprofloxacin)	Mean fluorescence/cell
1 day	
No drug	115.08 ± 4.1
80 µg/ml	120.87 ± 2.5
160 µg/ml	118.36 ± 1.0
3 days	
No drug	110.20 ± 9.8
80 µg/ml	103.56 ± 5.9
160 µg/ml	131.40 ± 2.4*

†Mouse L1210 cells continuously exposed to ciprofloxacin for 1 or 3 days were fixed with ethanol and the mean fluorescence/cell determined by FACS analysis at the University of Florida Flow Cytometry Core as described under Materials and Methods. The fluorescence was determined for 20,000 cells from each sample. The data were expressed as mean ±SE for three separate samples. Statistical analysis was performed using a one-way ANOVA with Student–Newman–Keul's post hoc test.
*$P < 0.05$.

than nalidixic acid (Fig. 5B). Treatment of cells with increasing concentrations of ciprofloxacin for 4 days resulted in a dose-dependent loss of mitochondrial respiration. Respiration was inhibited greater than 50% at 20 µg/ml ciprofloxacin. This drug also caused a dose-dependent decrease in COX activity with greater than 50% inhibition occurring at 80 µg/ml. This concentration was approximately 4-fold higher than that required to inhibit respiration, suggesting that the loss in respiration at 20 µg/ml ciprofloxacin was not due to a loss in COX activity. Possibly, the activities of other components that are important in mitochondrial respiration are more sensitive to the action of ciprofloxacin. In this regard, mtDNA encodes a number of genes for proteins which are important in mitochondrial energy metabolism. In addition to three subunits for COX, mtDNA encodes genes for apocytochrome b and subunits for cytochrome c, NADH dehydrogenase, and ATPase proteins (reviewed in Attardi and Shatz [1988]). Ciprofloxacin-induced depletion of mtDNA would therefore be expected to cause a decrease in the expression of a number of proteins important in energy metabolism. However, why a loss of mtDNA would affect the activities of some respiratory enzymes more than others is unclear but may be related to differences in the expression or the half-lives of these proteins.

Lactate Accumulates When Cells Are Treated With 4-Quinolones

The decrease in respiration following treatment of cells with 4-quinolones was associated with a corresponding increase in glycolysis as indicated by lactate accumulation (Fig. 6). Treatment of cells with 250 µg/ml nalidixic acid resulted in a time-dependent 3- to 4-fold increase in lactate accumulation that peaked by day 4 of

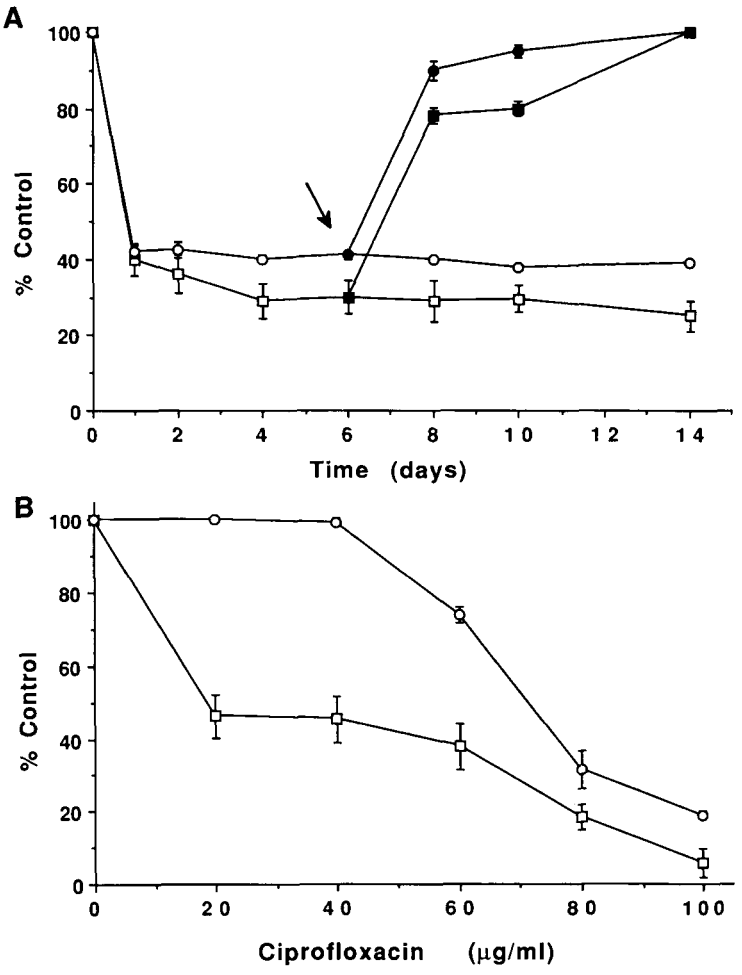

Fig. 5. Effects of nalidixic acid and ciprofloxacin on mitochondrial function. **A:** Oxygen consumption (□, ■) and cytochrome c oxidase activity (○, ●) were assayed in duplicate from mouse L1210 cells treated for various times with 250 μg/ml nalidixic acid as described under Materials and Methods. The closed symbols represent oxygen consumption and cytochrome c oxidase activities in cells that were removed from drug containing media at day 6 (marked by an arrow) and then placed in drug-free media for various lengths of time. **B:** Mouse L1210 cells were treated with various concentrations of ciprofloxacin for 4 days and the oxygen consumption (□) and cytochrome c oxidase activity (○) were assayed as described above. Activities were expressed as a percentage of the untreated control cells. Data represent the mean ±SE standard error of values obtained from six independent experiments.

the drug treatment (Fig. 6A). If cells were washed and then placed in drug-free media at day 6 (marked by an arrow), lactate accumulation returned to control levels within 4 days. The effect of 4-quinolones on lactate accumulation was dose-dependent. Continuous exposure of cells for 4 days to increasing concentrations of ciprofloxacin from 20–100 μg/ml resulted in as much as a 7-fold increase in lactate accumulation (Fig. 6B).

DISCUSSION

We have demonstrated that the 4-quinolone antibiotics, nalidixic acid, and ciprofloxacin, cause a selective loss of mtDNA from cultured mammalian cells. The loss of mtDNA was accompanied by a decrease in mitochondrial respiration and COX activity, followed by an inhibition of cell proliferation. These effects were reversible upon transfering cells to drug-free media with the recovery in mtDNA and respiration preceding the recovery in cell proliferation. These studies raise the interesting possibility that inhibition of growth by 4-quinolones is due to the depletion of mtDNA.

Ciprofloxacin and nalidixic acid are potent inhibitors of the bacterial type II topoisomerase DNA gyrase [Gellert et al., 1977; reviewed in

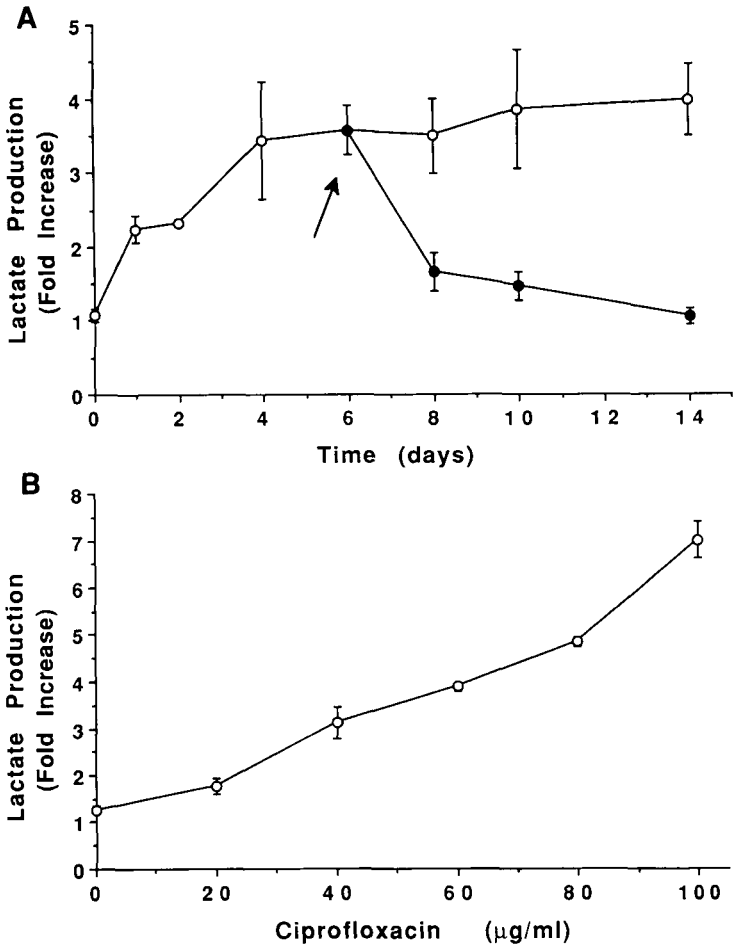

Fig. 6. Effect of nalidixic acid and ciprofloxacin on lactate accumulation. **A:** Lactate accumulation was measured in mouse L1210 cells treated with 250 μg/ml nalidixic acid (○) for various lengths of time as described under Materials and Methods. ●, lactate accumulation in cells removed from drug containing media at day 6 (marked by an arrow) and then placed in drug-free media for various lengths of time. **B:** Mouse L1210 cells were exposed to various concentrations of ciprofloxacin for 4 days and the lactate accumulation determined as described above. The amount of lactate was expressed relative to untreated control cells. Data represent the mean ±SE of values obtained from six independent experiments.

Drlica and Franco, 1988]. This enzyme plays an important role in removing topological problems that arise in bacterial DNA during replication (reviewed in Drlica and Franco [1988]). It has been hypothesized that a DNA gyrase-like type II topoisomerase is required to supercoil and decatenate mtDNA during the initiation and termination steps in mtDNA replication respectively [Clayton, 1982; Castora et al., 1982]. This is consistent with studies showing that mtDNA replication in isolated mitochondria could be arrested by the DNA gyrase inhibitors, nalidixic acid, and oxolinic acid [Castora et al., 1983]. The presence of a mitochondrial topoisomerase with properties similar to the bacterial enzyme DNA gyrase is not entirely surprizing, since mitochondria are thought to have originated in evolution as bacterial endosymbionts, accounting for many structural, biochemical, and genetic similarities of this organelle with bacteria [Gray, 1989]. Castora et al. [1982, 1985] partially purified a topoisomerase II activity from rat liver mitochondria that could catalyze the catenation and decatenation of plasmid DNA. However, this activity did not appear to be sensitive to the 4-quinolone drugs, nalidixic or oxolinic acid. More recently, Melendy and Ray [1989] have purified a mitochondrial topoisomerase II activity from the Trypanosomatid *Crithidia fasciculata* that was inhibited by concentrations of nalidixic acid that are effective against the bacterial enzyme DNA gyrase. This mitochondrial type II topoisomerase was also found to have physical and antigenic properties that were different from the

nuclear type II topoisomerase in this organism [Melendy and Ray, 1989]. Consistent with these studies, we have recently found that ciprofloxacin induces the formation of site-specific double-stranded breaks in mitochondrial but not nuclear DNA from Chinese hamster ovary cells (data not shown). We are currently investigating whether these breaks are mediated by a topoisomerase II-like protein in mitochondria.

There is some evidence which suggests that 4-quinolones may inhibit mammalian cell proliferation by interfering with the function of a nuclear topoisomerase II enzyme [Bredberg et al., 1991; Hussy et al., 1986]. However, it is puzzling why inhibition of nuclear topoisomerase II would cause a selective inhibition of mitochondrial but not nuclear DNA synthesis. Possibly, inhibition of a nuclear topoisomerase by 4-quinolones interferes with the transcription of nuclear-encoded mitochondrial proteins, resulting in the observed loss of mtDNA and mitochondrial functions. If this is true, other inhibitors of nuclear topoisomerase II should also deplete mtDNA from cells. Preliminary studies in our lab indicate that growth inhibition of mouse L1210 cells by the nuclear topoisomerase II-specific drug VM-26 [Liu, 1989] has no effect on mtDNA content, suggesting that the loss of mtDNA is not mediated through inhibition of a nuclear topoisomerase (data not shown).

Although a mitochondrial type II topoisomerase is a likely target for drug action, there are other activities that are required for mtDNA replication (e.g., DNA polymerases, helicases) that may also be inhibited by 4-quinolones. Drugs that are structurally unrelated to 4-quinolones, such as the DNA intercalator ethidium bromide, the polyamine analogue MGBG, and the dideoxynucleoside analogue ddC, have also been shown to cause a depletion of mtDNA from mammalian cells [Wiseman and Attardi, 1978; Nass, 1984; Vertino et al., 1991; Chen and Cheng, 1989]. The mechanisms underlying the depletion of mtDNA by ethidium bromide and MGBG are unknown. However, there is some evidence suggesting that ddC may be acting by inhibiting the mitochondrial enzyme DNA polymerase gamma [Chen and Cheng, 1989]. However, in vitro studies suggest that this enzyme is not inhibited by the 4-quinolone drugs nalidixic acid or oxolinic acid [Castora et al., 1983]. We are currently attempting to isolate a drug-sensitive topoisomerase activity from mammalian mitochondria. These studies should help establish whether the selective depletion of mtDNA by 4-quinolones is due to inhibition of a mitochondrial topoisomerase.

ACKNOWLEDGMENTS

We thank Melissa Chen and Neil Benson in the Flow Cytometry Core Laboratory at the University of Florida for their assistance with the FACS analysis. This work was supported by Grant F9OUF-3 from the Florida Division of the American Cancer Society.

REFERENCES

Attardi G, Shatz G (1988): Biogenesis of mitochondria. Annu Rev Cell Biol 4:289–333.

Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA (1981): Sequence and gene organization of mouse mitochondrial DNA. Cell 26:167–180.

Bredberg G, Brent M, Jaszyk M (1991): Ciprofloxacin-induced inhibition of topoisomerase II in human lymphoblastoid cells. Antimicrob Agents Chem 35:448–450.

Castora FJ, Sternglanz R, Simpson MV (1982): In Attardi G, Slonimski P, Borst P (eds): "Mitochondrial Genes." Cold Spring Harbor, New York: Cold Spring Harbor Press, pp 143–154.

Castora FJ, Vissering FF, Simpson MV (1983): The effects of bacterial DNA gyrase inhibitors on DNA synthesis in mammalian mitochondria. Biochim Biophys Acta 740:417–427.

Castora FJ, Lazarus GM, Kunes D (1985): The presence of two mitochondrial DNA topoisomerases in human acute leukemia cells. Biochem Biophys Res Commun 130:854–866.

Chen C-H, Cheng Y-C (1989): Delayed cytotoxicity and selective loss of mitochondrial DNA in cells treated with the anti-human immunodeficiency virus compound 2′,3′-dideoxycytidine. J Biol Chem 264:11934–11937.

Clayton DA (1982): Replication of animal mitochondrial DNA. Cell 28:693–705.

Deininger PL, Jolly DJ, Rubin CM, Friedmann T, Schmid CW (1981): Base sequence studies of 300 nucleotide renatured repeated human DNA clones. J Mol Biol 151:17–33.

Desjardins P, Frost E, Morais R (1985): Ethidium bromide-induced loss of mitochondrial DNA from primary chicken embryo fibroblasts. Mol Cell Biol 5:1163–1169.

Drlica K, Franco RJ (1988): Inhibitors of DNA topoisomerases. Biochemistry 27:2253–2259.

Esnault C, Brown SC, Segal-Bendirdjian E, Coulaud D, Misha Z, Roques BP, Le Pecq JB (1990): Selective alteration of mitochondrial function by ditercalinium (NCS 33153), a DNA bisintercalating agent. Biochem Pharmacol 390:109–122.

Gellert M, Mizzuuchi K, O'Dea MH, Itoh T, Tomizawa J (1977): Nalidixic acid resistance: A second genetic character involved in DNA gyrase activity. Proc Natl Acad Sci USA 74:4772–4776.

Gootz TD, Barrett JF, Sutcliffe JA (1990): Inhibitory effects of quinolone antibacterial agents on eukaryotic topoisomerases and related test systems. Antimicrob Agents Chem 34:8–12.

Gray MW (1989): Origin and *evolution of mitochondrial* DNA. Annu Rev Cell Biol 5:25–50.

Hauswirth WW, Lim LO, Dujon B, Turner G (1987): In Darley-Usmar VM, Rickwood D, Wilson MT (eds): "Mitochondria: A Practical Approach." Washington, DC: IRL Press, pp 171–244.

Hussy P, Maass G, Tummler B, Grosse F, Schombury U (1986): Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase alpha primase complex, topoisomerase I and II, and growth of mammalian lymphoblasts. Antimicrob Agents Chem 29:1073–1078.

Kroeger PE, Rowe TC (1989): Interaction of topoisomerase I with the transcribed region of the *Drosophila* HSP 70 heat shock gene. Nucleic Acids Res 17:8495–8509.

Lebarca C, Paigen K (1980): A simple, rapid, and sensitive DNA assay procedure. Anal Biochem 102:344–352.

Liu LF (1989) DNA topoisomerases poisons as antitumor drugs. Annu Rev Biochem 58:351–375.

Melendy T, Ray DS (1989): Novobiocin affinity purification of a mitochondrial type II topoisomerase from the trypansomatid *Crithidia fasciculata*. J Biol Chem 264:1870–1876.

Nass MMK (1984): Analysis of methylglyoxal bis(guanylhydrazone)-induced alterations of hamster tumor mitochondria by correlated studies of selective rhodamine binding, ultrastructure damage, DNA replication, and reversibility. Cancer Res 44:2677–2688.

Oomori Y, Yasue T, Aoyama H, Hirai K, Suzue S, Yokota T (1988): Effects of fleroxacin on HeLa cell functions and topoisomerase II. J Antimicrob Agents Chem 22(suppl D):91–97.

Sambrook J, Fritsch EF, Maniatis T (1989): "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, pp 9.41–9.55.

Segal-Bendirdjian E, Coulaud D, Roques BP, LePecq JB (1988): Selective loss of mitochondrial DNA after treatment of cells with ditercalinium (NSC335153), an antitumor bis-intercalating agent. Cancer Res 48:4982–4992.

Shin H-S, Bargiello TA, Clark BT, Jackson FR, Young MW (1985): An unusual coding sequence from a *Drosophila* clock gene is conserved in vertebrates. Nature 317:445–448.

Southern EM (1975): Detection of specific sequences among DNA fragments by gel electrophoresis. J Mol Biol 98:503–517.

Tzagoloff A (1982): "Mitochondria." New York: Plenum Press, pp 113–116.

Vertino PM, Beerman TA, Kelly EJ, Bergeron RJ, Porter CW (1991): Selective cellular depletion of mitochondrial DNA by the polyamine analog N^1,N^{12}-bis(ethyl)spermine and its relationship to polyamine structure and function. Mol Pharmacol 39:487–494.

Wiseman A, Attardi G (1978): Reversible tenfold reduction in mitochondrial DNA content of human cells treated with ethidium bromide. Mol Gen Genet 167:51–63.