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Effects of Ciprofloxacin on Eucaryotic Pyrimidine Nucleotide Biosynthesis and Cell Growth

ARNE FORSGREN,[1,2]* ANDERS BREDBERG,[2] ARTHUR B. PARDEE,[3] STUART F. SCHLOSSMAN,[1] AND THOMAS F. TEDDER[1]

Divisions of Tumor Immunology[1] and Cell Growth and Regulation,[3] Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, and Department of Medical Microbiology, University of Lund, Malmö General Hospital, S-2140 Malmö, Sweden[2]

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Several of the new 4-quinolones significantly increase the incorporation of [^{3}H]thymidine into the DNA of mitogen-stimulated human lymphocytes. This study suggests that ciprofloxacin inhibits de novo pyrimidine biosynthesis, thereby resulting in a compensatory increase in the uptake of pyrimidine precursors through salvage pathways, and that additional effects may affect eucaryotic cell growth. Incorporation of deoxyuridine, uridine, and orotic acid as well as thymidine was increased in the presence of ciprofloxacin, one of the antibacterially most active of the new 4-quinolones. In contrast, the uptake was decreased in very high concentrations of the drug. Culture in HAT (hypoxanthine, aminopterine, thymidine) medium, which blocks de novo thymidylate synthesis, abrogated the increase in [^{3}H]thymidine incorporation induced by ciprofloxacin. Ciprofloxacin also failed to increase the uptake of [^{14}C]hypoxanthine or leucine, indicating a selective effect on pyrimidine and not on purine nucleotide biosynthesis. *N*-(Phosphonacetyl)-L-aspartate, an inhibitor of pyrimidine nucleotide biosynthesis, also increased [^{3}H]thymidine incorporation in phytohemagglutinin-stimulated lymphocytes in a fashion similar to ciprofloxacin. The growth of several cell lines was partially inhibited by ciprofloxacin at 20 μg/ml and completely inhibited at 80 to 160 μg/ml. Growth inhibition by ciprofloxacin could not be restored by the addition of uridine to the medium. Chromosome breaks, gene amplification, or other genetic alterations could not be detected in human lymphocytes incubated with up to 25 μg of ciprofloxacin per ml.

Many of the new 4-quinolones have a broader spectrum and are more potent than their structurally related predecessors (7, 12). The antibacterial activity of nalidixic acid is caused by inhibition of DNA synthesis, resulting from inhibition of DNA gyrase (23, 28). Although the new 4-quinolones have not been studied as extensively as nalidixic acid, they have been shown to inhibit gyrase function (2, 10, 21, 22), possibly through DNA binding (19). However, a recent study demonstrated a lack of correlation between antibacterial activity and inhibition of gyrase activity, thereby suggesting that other factors are responsible for the potency of these drugs (29).

We have found that in eucaryotic cells, many of the new quinolones cause a significant (11 to 231%) increase in the uptake of [^{3}H]thymidine into trichloroacetic acid (TCA)-precipitable material in mitogen-stimulated human lymphocytes at drug concentrations of 1.6 to 50 μg/ml (8; A. Forsgren, S. F. Schlossman, and T. F. Tedder, submitted for publication). However, cell growth and progression through the cell cycle were inhibited by 20 μg or more of ciprofloxacin per ml. In addition, ciprofloxacin at a clinically achievable concentration of 5 μg/ml caused a 50% decrease in immunogloblin production by mitogen-stimulated lymphocytes in vitro.

In this study, we investigated the reasons for the increased [^{3}H]thymidine uptake and the decreased function of lymphocytes cultured in the presence of ciprofloxacin. We found that ciprofloxacin may block pyrimidine but not purine metabolism, thereby resulting in a compensatory increased uptake of pyrimidine nucleotide precursors through salvage pathways. Additionally, other effects on eucaryotic cells may inhibit cell growth.

MATERIALS AND METHODS

Antimicrobial agents. Ciprofloxacin (lot no. 907337) was kindly supplied by Bayer, Wuppertal, Federal Republic of Germany, and *N*-(phosphonacetyl)-L-aspartate (PALA) (1, 24) was a gift from Clarence Fortner, National Cancer Institute. The drugs were prepared daily in sterile RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10 to 15% fetal calf serum.

Cells and cell culture. Human lymphocytes were obtained from heparinized blood by centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) and then washed twice in RPMI 1640 medium containing 5% fetal calf serum. Human lymphocytes (10^5) were cultured in triplicate wells of 96-well microtiter plates each containing 200 μl of RPMI 1640 medium supplemented with 15% fetal calf serum, 2 mM glutamine, and 50 μg of gentamicin per ml. Phytohemagglutinin (PHA) (Wellcome Research Laboratories, Beckenham, England) was added to a final concentration of 1 μg/ml, which gave maximal stimulation. In some experiments, lymphocytes were grown in this medium supplemented with hypoxanthine (Sigma Chemical Co., St. Louis, Mo.) at 13.6 μg/ml, aminopterin (Sigma) at 0.18 μg/ml, and thymidine (Sigma) at 0.39 μg/ml (HAT medium).

The human lymphoblastoid cell line Raji was cultured in RPMI 1640 medium containing 10% fetal calf serum, glutamine, and gentamicin. Chinese hamster ovary cells CHO-K1, the mouse embryo fibroblast cell lines 3T3 and 3T6, HeLa cells, and cells from the normal human fibroblast cell line FS-2 were grown in Dulbecco minimal essential medium supplemented with 10% fetal calf serum, 2 mM

* Corresponding author.

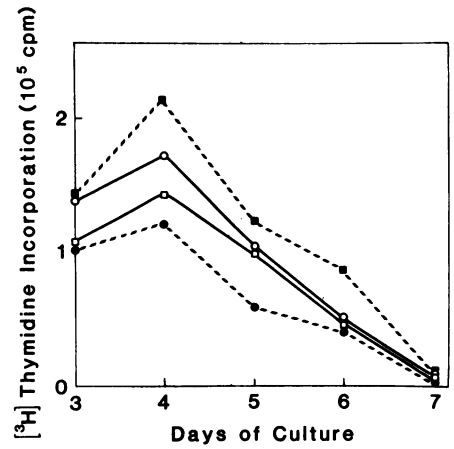

FIG. 1. Incorporation of [^{3}H]thymidine by PHA-stimulated human lymphocytes cultured in HAT medium with (□) or without (○) 5 μg of ciprofloxacin per ml or in regular RPMI medium with (■) or without (●) 5 μg of ciprofloxacin per ml. Cultures were pulsed with [^{3}H]thymidine 16 to 18 h before being harvested. Values represent the mean counts per minute for triplicate cultures, and standard deviations were less than 15%. These results are representative of those obtained in four different experiments.

L-glutamine, and penicillin-streptomycin (M.A. Bioproducts, Walkersville, Md.). These cells were cultured in a volume of 1 ml in flat-bottom 24-well plates (GIBCO) with an area per well of approximately 2.0 cm^2 or in 10 ml in 25-cm^2 tissue culture flasks. Adherent cells were detached from the culture vessel with a trypsin-versene mixture (M.A. Bioproducts) and counted in a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.). In some experiments, the cultures were supplemented with 5×10^{-6} to 5×10^{-4} M uridine (Sigma). Test drugs were added to adherent cell cultures 24 h after the initiation of culture to test their effects on exponentially growing cells.

Radioactive incorporation. A 0.5- or 1-μCi amount of [^{3}H]thymidine (NET-0272; specific activity, 78 Ci/mmol [New England Nuclear Corp., Boston, Mass.]), [6-^{3}H]deoxyuridine (NET-164; specific activity, 21.4 Ci/mmol), [^{3}H]uridine (NET 367; specific activity, 40 Ci/mmol), [5-^{3}H]orotic acid (NET-134; specific activity, 10 to 20 Ci/mmol), [8-^{14}C]hypoxanthine (NEC-149; specific activity, 40 to 60 mCi/mmol), or [^{14}C]leucine (NEC-279; specific activity, 337 mCi/mmol) was added to cell cultures 6 to 24 h before harvesting. Cultures were usually harvested in distilled water onto glass fiber filters (Skatron A/S, Liberbyen, Norway) by an automatic harvesting machine. In some experiments, the filters were carefully washed with 10% TCA after the cultures were harvested. Incorporation of [^{14}C]leucine was determined by first pelleting the cells and then suspending them in a small volume of saline. The suspension was mixed with an equal volume of 20% TCA. After 20 min at 0°C, the resulting acid-precipitated material was collected on a glass fiber filter and carefully washed with 10% TCA and finally with 95% ethanol. The filters were dried and transferred to scintillation vials containing 2 ml of Insta-Fluor solution (Packard Instrument Co., Inc., Rockville, Md.). The radioactivity was measured in a Packard Tri-Carb liquid scintillation counter, and data were calculated from the means of triplicate cultures.

Analysis of DNA damage. Chromosome preparations from lymphocyte cultures were treated with trypsin before being stained with Giemsa to produce banding patterns (26). The alkaline elution method of Kohn (13) was used to study DNA strand breaks. Epstein-Barr virus-transformed human lymphoblasts from a normal donor were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum. [^{14}C]thymidine (final concentration, 0.1 μCi/ml) was present for 24 h before a 2-h chase period. The cells (0.4 ml at 2×10^6 cells per ml) were then pipetted onto a filter (pore size, 2 μm; Nuclepore Corp., Pleasanton, Calif.) and lysed with a 2% sodium dodecyl sulfate–20 mM EDTA (pH 10)–0.5-mg/ml proteinase K solution. Finally, tetrapropylammonium hydroxide (pH 12) was slowly pumped through the filter, and 5-ml fractions were collected, mixed with 2 volumes of Aquasol (New England Nuclear), and measured by liquid scintillation counting. Activity remaining on the filter was recovered as described by Kohn (13). The lymphoblasts were exposed, while kept on ice, to UV radiation (predominantly 254 nm) from an unfiltered germicidal lamp (G15T8; General Electric Co., Schenectady, N.Y.) at a dose rate of 0.1 J/m^2 per s.

Statistics. Wilcoxon's matched pair test was used to check the statistical significance of the results.

RESULTS

Incorporation of radioactive DNA, RNA, and protein precursors in the presence of ciprofloxacin. Quinolones enhance the incorporation of [^{3}H]thymidine by mitogen-stimulated human lymphocytes (8). In HAT culture medium, nucleic acid metabolism is dependent on the uptake of thymidine and hypoxanthine through salvage pathways, since de novo thymidine and purine nucleotide biosynthesis is blocked by aminopterin. There was no increase in thymidine incorporation by cells cultured in HAT medium in the presence of 5 μg of ciprofloxacin per ml, in contrast to the situation in regular medium (Fig. 1). Instead, there was a slight decrease in incorporation in most experiments. Similar results were obtained with 20 μg of ciprofloxacin per ml. These results suggest that ciprofloxacin affects de novo pyrimidine biosynthesis, resulting in increased uptake of radiolabeled thymidine through salvage pathways.

To determine whether increased incorporation of other nucleotides or amino acids occurs, the incorporation of radiolabeled thymidine, deoxyuridine, uridine, and leucine in parallel cultures of PHA-stimulated human lymphocytes during 1- to 6-day cultures was studied. Radiolabeled precursors were added during the last 8 to 18 h of incubation. Cultures harvested after 24 h gave less than 5,000 cpm of incorporated radioactivity. During day 2 of culture, the uptake of radiolabeled precursors was low but significantly ($P < 0.01$) inhibited by ciprofloxacin at concentrations of 20 μg/ml or higher (Fig. 2). However, from day 3 to day 5 of incubation, the incorporation of radiolabeled thymidine, deoxyuridine, and uridine was enhanced by the presence of ciprofloxacin, although the increased uptake of uridine was less pronounced (Fig. 2). At a ciprofloxacin concentration of 80 μg/ml, incorporation was strongly inhibited. The incorporation of [^{14}C]leucine was not significantly changed from that in control cultures at ciprofloxacin concentrations up to 20 μg/ml, while at 40 and 80 μg/ml, leucine incorporation was inhibited. Similar results were obtained when the cell material was precipitated and washed with TCA, demonstrating radioactive precursor incorporation into nucleic acids and protein.

When the total radioactivity incorporated over a 6-day period was determined by daily pulsing and harvesting, it was found that at 20 μg of ciprofloxacin per ml, [^{3}H]thy-

midine and [^{3}H]deoxyuridine incorporation was increased by 42 to 45%, [^{3}H]uridine uptake was increased by 27%, and leucine incorporation was decreased by 18% (Table 1). At 5 μg/ml, a similar effect was observed.

Thymidine, deoxyuridine, and uridine are incorporated through salvage pathways into steps of de novo pyrimidine nucleotide biosynthesis later than orotate biosynthesis. The uptake of orotic acid was also increased (8 to 56%) by ciprofloxacin, consistent with inhibition by the drug of an early step in pyrimidine synthesis (Table 2). Hypoxanthine is a precursor of purines in DNA synthesis. The incorporation of radiolabeled hypoxanthine was not increased by ciprofloxacin, in contrast to the incorporation of thymidine (Fig. 3). The inhibition thus appears to affect pyrimidine and not purine pathways.

Increase of [^{3}H]thymidine uptake by PALA. PALA, a transition state analog inhibitor of aspartate transcarbamylase, has been shown to cause marked reductions in the uracil nucleotide pools of cells by inhibiting de novo pyrimidine biosynthesis. PALA at 15 to 60 μg/ml increases and at high doses (240 and 480 μg/ml) inhibits the incorporation of [^{3}H]thymidine into PHA-stimulated lymphocytes in a similar fashion to ciprofloxacin (Table 3).

Cell growth. Quinolones also affect cell growth of mitogen-stimulated lymphocytes (Forsgren et al., submitted). The growth of HeLa cells was also significantly inhibited ($P < 0.01$) at 20 μg of ciprofloxacin per ml, and there was no growth at all at 80 μg of the drug per ml (Fig. 4a). Similar results were obtained with the mouse fibroblast cell line 3T3 and the human fibroblast cell line FS-2. The Chinese hamster ovary cell line CHO-K1 and the mouse embryo fibroblast cell line 3T6 were slightly more resistant to ciprofloxacin, i.e., complete growth inhibition was obtained only at 128 μg of ciprofloxacin per ml. The inhibitory effect of ciprofloxacin on HeLa cell growth was reversible (Fig. 4b). The growth of cells exposed to 80 μg of the drug per ml for 24 h and then washed was not inhibited. PALA inhibited the growth of CHO-K1 cells, with strong inhibition detected at 160 μg/ml. The addition of 3×10^{-4} M uridine completely restored growth. However, when the growth of PHA-stimulated lymphocytes, CHO-K1, or 3T6 cells was partially inhibited at 20 μg or completely inhibited at 80 to 160 μg of ciprofloxacin per ml, the addition of uridine up to 5×10^{-4} M did not restore growth.

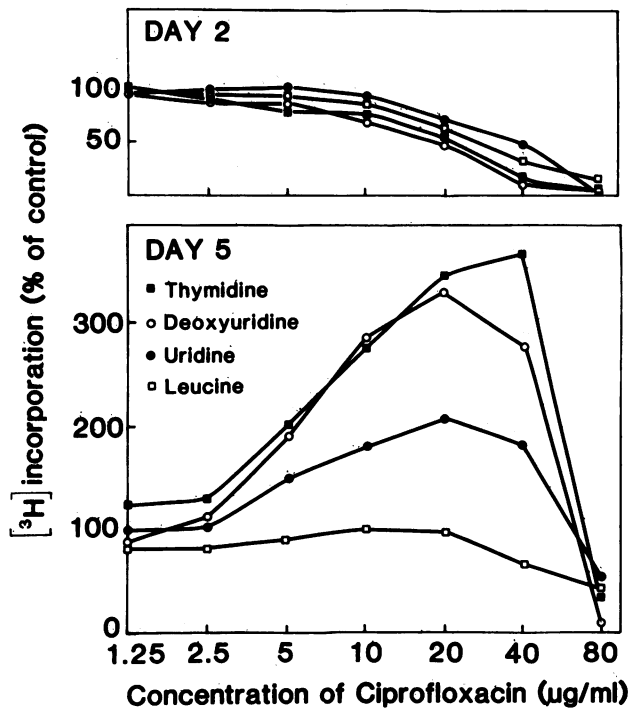

FIG. 2. Incorporation of [^{3}H]thymidine, [^{3}H]deoxyuridine, [^{3}H]uridine, and [^{14}H]leucine by PHA-stimulated lymphocytes in the presence of different concentrations of ciprofloxacin. Cultures were pulsed 16 to 18 h before being harvested after 2 and 5 days, respectively. The incorporations of [^{3}H]thymidine, [^{3}H]deoxyuridine, [^{3}H]uridine, and [^{14}C]leucine in the controls without ciprofloxacin were 108,658, 29,122, 53,265, and 10,451 cpm and 157,971, 83,046, 58,638 and 20,973 cpm after 2 and 5 days, respectively. These results are representative of those obtained in five independent experiments.

TABLE 1. Influence of ciprofloxacin on the total incorporation of nucleosides (but not leucine) during 6 days of culture

Nucleoside or amino acid	% of control ± SD at drug concn (μg/ml) of[a]:		
	20	5	1.25
[^{3}H]thymidine	145 ± 15	142 ± 6	110 ± 4
[^{3}H]deoxyuridine	142 ± 21	139 ± 5	102 ± 2
[^{3}H]uridine	127 ± 11	119 ± 9	110 ± 3
[^{14}C]leucine	82 ± 14	96 ± 7	92 ± 3

[a] Human lymphocytes were stimulated with PHA and incubated with ciprofloxacin. The cultures were pulsed with radioactive nucleosides or amino acid before being harvested at 24-h intervals on days 1 to 6. Incorporation (counts per minute) for each day was totaled, and the results were calculated as a percentage of control cultures not containing ciprofloxacin and are the mean ± standard deviation of results obtained in three independent experiments.

Chromosome and macromolecular DNA studies. The banding pattern of chromosomes isolated from lymphocytes cultured with up to 40 μg of ciprofloxacin per ml for 1 to 5 days was examined microscopically. A detectable increase in the frequency of breaks, amplifications, or other chromosomal aberrations due to drug treatment was not observed.

DNA strand breaks in Epstein-Barr virus-transformed human lymphocytes were assayed directly by using the Kohn alkaline elution technique (13) (Fig. 5). In these experiments, ciprofloxacin was present between 1 and 48 h (only 24 h shown in Fig. 1) and until sodium dodecyl sulfate lysis of the cells on the elution filter. Labeled thymidine was added 24 h before cell lysis. In principle, the presence of breaks in the DNA would decrease the size of the fragments of DNA on the filter, leading to an increased elution rate. DNA strand breakage in human lymphoblasts was shown by alkaline elution only at quinolone concentrations of 100 μg/ml (Fig. 5). Exposure to 0.5, 2, 10 and 25 μg of ciprofloxacin per ml resulted in a normal elution rate. Cells irradiated with 300 rads of X rays or exposed to UV light were included as internal standards. This X-ray dose caused a marked increase in the elution rate and can be estimated to yield about 600 DNA strand breaks per cell, corresponding to DNA fragments of molecular weight 2×10^9, indicating the high sensitivity of this method. Cells assayed immediately after shortwave UV exposure (cells were kept on ice during the irradiation procedure) did not reveal an elevated level of DNA breaks, whereas increased DNA breakage occurred during a post-UV incubation period at 37°C (Fig. 5). These UV-dependent DNA breaks are usually referred to as part of a DNA repair process leading to excision of UV-damaged nucleotides from the cellular DNA (6, 9). The presence of ciprofloxacin at a concentration of 10 μg/ml (X rays) or 5 μg/ml (UV) during exposure of the cells to

TABLE 2. [^{3}H]orotic acid and [^{3}H]thymidine incorporation in PHA-stimulated human lymphocytes cultured with and without ciprofloxacin[a]

Day of culture	[^{3}H]orotic acid incorporation (cpm) ± SD for:			[^{3}H]thymidine incorporation (cpm) ± SD for:		
	No ciprofloxacin	Ciprofloxacin (5 μg/ml)	% of control	No ciprofloxacin	Ciprofloxacin (5 μg/ml)	% of control
3	1,068 ± 235	1,164 ± 160	109	69,142 ± 6,777	98,160 ± 12,147	142
4	2,449 ± 498	3,288 ± 288	134	92,467 ± 14,239	137,050 ± 7,216	148
5	2,102 ± 69	3,270 ± 393	156	102,378 ± 7,268	151,110 ± 4,887	148

[a] Values represent the mean ± standard deviation of triplicate cultures. These results are representative of those obtained in five similar experiments.

radiation did not noticeable affect the resulting elution curves. These data indicate that ciprofloxacin does not influence the frequency of breaks generated during exposure to X rays or the formation of breaks during enzymatic DNA repair of UV damage.

DISCUSSION

Exogenous thymidine, deoxyuridine, uridine, and orotic acid are precursors of pyrimidine nucleotides in different salvage pathways. Each of these precursors was incorporated at higher levels by cells cultured in the presence of ciprofloxacin (Tables 1 and 2; Fig. 2). However, in HAT medium, in which de novo pyrimidine synthesis is blocked, no increase in [^{3}H]thymidine uptake due to ciprofloxacin was detected (Fig. 1). Incorporation of the purine hypoxanthine or of the amino acid leucine were not increased in the presence of ciprofloxacin (Table 1; Fig. 3). These data suggest that ciprofloxacin inhibits de novo pyrimidine synthesis at a step before orotate synthesis. Such an inhibition could result in the increased uptake of pyrimidine nucleotide precursors. This was supported by results from experiments with PALA, a well-defined transition state analog inhibitor of aspartate transcarbamylase (1, 20). PALA inhibition occurs at a point before orotate synthesis in the pyrimidine biosynthetic pathway, causing marked reductions in the nucleotide pools by inhibiting de novo pyrimidine biosynthesis (14, 17, 24). In our experiments with PHA-stimulated lymphocytes, PALA caused an increased incorporation of [^{3}H]thymidine in a similar way to that caused by several of the new 4-quinolones (Table 3). This finding strengthens the hypothesis that the new quinolones at low concentrations inhibit de novo pyrimidine biosynthesis by mammalian cells in vitro.

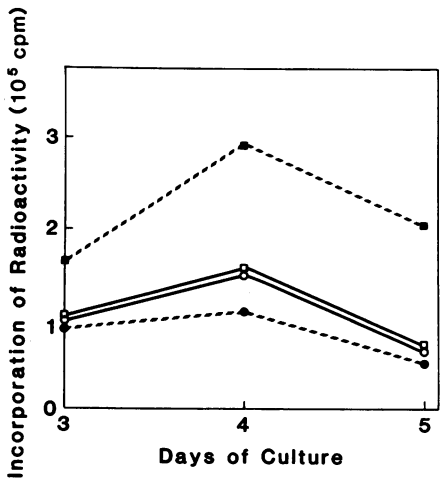

FIG. 3. Incorporation of [^{14}C]hypoxanthine by PHA-stimulated lymphocytes in the presence (□) or absence (○) of 5 μg of ciprofloxacin per ml and [^{3}H]thymidine in the presence (■) or absence (●) of 5 μg of ciprofloxacin per ml. These results are representative of those obtained in five independent experiments. Values represent the mean counts per minute for triplicate cultures, and standard deviations were less than 15%.

Normal human lymphocytes and numerous mammalian cell lines were partially inhibited in their growth by 20 μg of ciprofloxacin per ml and completely inhibited at 80 to 160 μg/ml (Fig. 4). When the media were supplemented with uridine to provide precursors for nucleotide biosynthesis through salvage pathways, growth in the presence of 20 to 160 μg of ciprofloxacin per ml was not restored by the supplement. In contrast, when growth of the Chinese hamster cell line CHO-K1 was completely inhibited by PALA, it could be restored by the addition of 3×10^{-4} M uridine to the medium. This suggests that in addition to a possible inhibiting effect on pyrimidine metabolism, ciprofloxacin may have other effects on mammalian cells, leading to growth inhibition in vitro.

The A subunits of bacterial DNA topoisomerase II (DNA gyrase) introduce single- or double-strand incisions at specific sites on each DNA strand and reseal such breaks (4). The primary action of nalidixic acid has been suggested to be prevention of the resealing of these breaks, causing the death of bacteria or induction of mutagenic SOS repair functions (4, 27). This effect could also affect mammalian cells. However, the theory that the major antibacterial effect of the new 4-quinolones is through action on topoisomerase II (21) has been seriously challenged (29). The induction of DNA breaks with repair could explain the increased incorporation of [^{3}H]thymidine in the presence of ciprofloxacin at low concentrations. In most cell types, the amount of DNA synthesized during excision repair involves replacement of small segments of the genome. [^{3}H]thymidine incorporation into DNA as a result of excision repair would therefore contribute little to the total thymidine uptake in a proliferating cell population (15). Also, alkaline elution did not reveal any DNA breaks caused by the new quinolones. Thus it

TABLE 3. [^{3}H]thymidine incorporation by PHA-stimulated lymphocytes cultured with PALA

Concn of drug (μg/ml)	[^{3}H]thymidine incorporation (cpm ± SD [% of control]) on[a]:	
	Day 3	Day 5
0	89,176 ± 1,947	46,606 ± 8,662
15	138,554 ± 2,693 (155)	88,766 ± 13,822 (190)
30	164,548 ± 7,580 (185)	103,732 ± 8,423 (223)
60	127,006 ± 9,961 (142)	74,894 ± 2,195 (161)
120	93,457 ± 10,061 (105)	51,187 ± 5,876 (110)
240	69,098 ± 3,375 (77)	48,243 ± 7,092 (104)
480	56,879 ± 440 (64)	28,062 ± 4,582 (60)

[a] Values represent the mean ± standard deviation of triplicate cultures. These results are representative of those obtained in three experiments.

seems unlikely that DNA repair is the sole explanation for the dramatically increased uptake of [^{3}H]thymidine in PHA-stimulated lymphocytes in the presence of low concentrations of the new quinolones.

Gene amplification as a mechanism for overproduction of specific proteins in cultured somatic cells has been an increasingly reported phenomenon (16) and could result in increased thymidine incorporation. However, chromosome-banding studies did not reveal any amplifications or elevated levels of other abberrations in the chromosome structure of lymphocytes cultured in the presence of ciprofloxacin. Novobiocin is an inhibitor of the ATPase subunit of bacterial and eucaryotic type II topoisomerases and DNA polymerase α and affects the topoisomerases of mitochondria (3, 5). The new quinolones might also inhibit mitochrondrial function, resulting in an inhibited progression through the cell cycle. Another explanation for increased [^{3}H]thymidine uptake caused by ciprofloxacin could be an inhibition of thymidylate synthase. In fact, it has been suggested that inhibitors of topoisomerase II, ribonucleoside reductase, or DNA polymerase are also indirect inhibitors of thymidylate synthetase (25). However, the increased uptake of deoxyuridine, uridine, and orotic acid in the presence of ciprofloxacin cannot be explained by that mechanism.

At present, little is known about the effects of the new

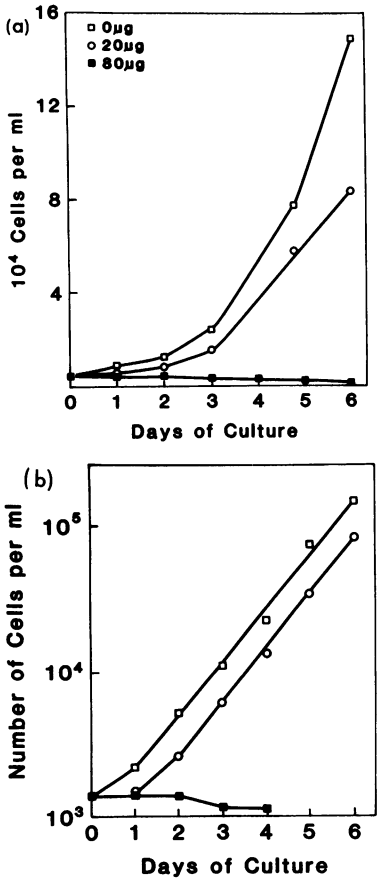

FIG. 4. (a) Growth of HeLa cells in the presence of 0 (□), 20 (○), and 80 μg (■) of ciprofloxacin per ml. (b) HeLa cells were cultured with 80 μg of ciprofloxacin per ml for 24 h, washed, and cultured with 80 μg of ciprofloxacin per ml (■) or without ciprofloxacin (○). Control cells (□) were cultured without ciprofloxacin. These results are representative of those obtained in five experiments.

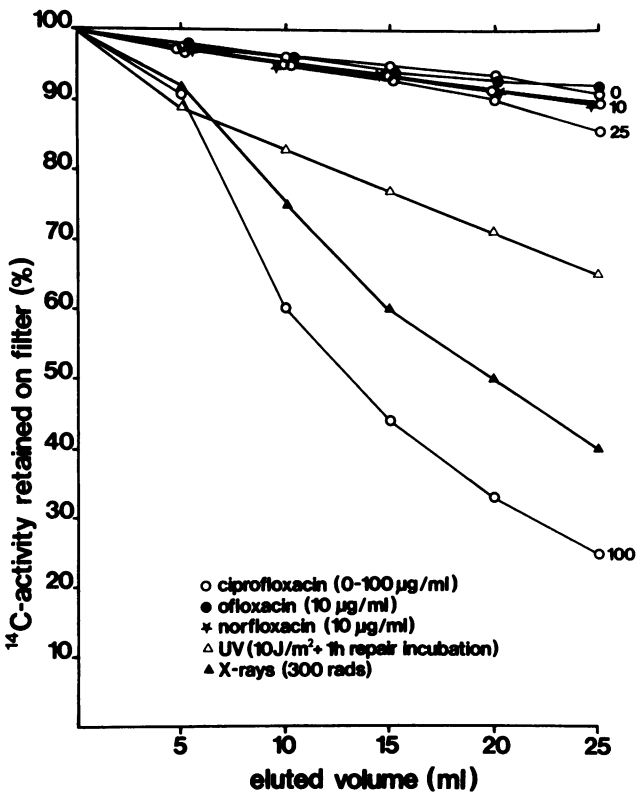

FIG. 5. Analysis of DNA strand breaks in Epstein-Barr virus-transformed human lymphoblasts exposed to ciprofloxacin, norfloxacin, and ofloxacin for 24 h. The cells were labeled with [^{14}C]thymidine, and strand breaks were analyzed by the alkaline elution technique of Kohn (13). Elution profiles of cells exposed to X rays and shortwave UV, respectively, are included to indicate the level of sensitivity of the assay.

4-quinolone derivatives on mammalian cellular metabolism, but at high concentrations they have been reported to be capable of inhibiting eucaryotic DNA polymerases α and β and deoxynucleotidyltransferase in cell-free systems (18). A significant decrease in the amount of DNA synthesized in mammalian cells was observed only with concentrations of more than 100 μg of the new 4-quinolones per ml, and DNA polymerase α and topoisomerases I and II were inhibited only at high concentrations of the drugs (11). Our results suggest that the quinolone drugs at low concentrations may also affect de novo pyrimidine biosynthesis and at high concentrations may inhibit cell growth in vitro. Altered pyrimidine biosynthesis in the presence of 4-quinolones may represent a specific inhibition of one of the enzymes involved in biosynthesis. However, a less specific or another toxic effect of 4-quinolones may occur, since the effect seen occurs after considerable delay. The uncertainty surrounding the actual mode of action of the 4-quinolones in mammalian cells and in bacteria suggests that further studies comparing the biological effects of the 4-quinolones with their behavior in biochemical assays are necessary to resolve the complexities of their mode of action.

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