ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1987, p. 768–773
0066-4804/87/050768-06$02.00/0

Vol. 31, No. 5

4-Quinolone Drugs Affect Cell Cycle Progression and Function of Human Lymphocytes In Vitro

ARNE FORSGREN,[1,2]* STUART F. SCHLOSSMAN,[1] AND THOMAS F. TEDDER[1]

The Division of Tumor Immunology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115,[1] and The Department of Medical Microbiology, University of Lund, Malmö General Hospital, Malmö, Sweden[2]

Received 4 August 1986/Accepted 17 February 1987

Most antibacterial agents do not affect human lymphocyte function, but a few are inhibitory. In contrast, a pronounced increase in the incorporation of [^{3}H]thymidine in the presence of 4-quinolones was observed in these studies. The uptake of [^{3}H]thymidine into DNA (trichloroacetic acid precipitable) was significantly increased in phytohemagglutinin-stimulated human lymphocytes when they were exposed to eight new 4-quinolone derivatives, ciprofloxacin, norfloxacin, ofloxacin, A-56619, A-56620, amifloxacin, enoxacin, and pefloxacin, at 1.6 to 6.25 μg/ml for 5 days. Four less antibacterially active 4-quinolones (nalidixic acid, cinoxacin, flumequine, and pipemidic acid) stimulated [^{3}H]thymidine incorporation only at higher concentrations or not at all. Kinetic studies showed that incorporation of [^{3}H]thymidine was not affected or slightly inhibited by ciprofloxacin 2 days after phytohemagglutinin stimulation but was increased on days 3 to 6. The total incorporation of [^{3}H]thymidine from day 1 to day 6 after phytohemagglutinin stimulation was increased by 42 to 45% at 5 to 20 μg of ciprofloxacin per ml. Increased [^{3}H]thymidine incorporation was also seen when human lymphocytes were stimulated with mitogens other than phytohemagglutinin. Ciprofloxacin added at the start of the culture had a more pronounced effect on [^{3}H]thymidine incorporation than when added later. In spite of the apparent increase in DNA synthesis, lymphocyte growth was inhibited by 20 μg of ciprofloxacin per ml, and cell cycle analysis showed that ciprofloxacin inhibited progression through the cell cycle. In addition, immunoglobulin secretion by human lymphocytes stimulated by pokeweed mitogen or Epstein-Barr virus was inhibited by approximately 50% at 5 μg of ciprofloxacin per ml. These results suggest that the 4-quinolone drugs may also affect eucaryotic cell function in vitro, but additional studies are needed to establish an in vivo relevance.

The new 4-quinolones such as ciprofloxacin, norfloxacin, and ofloxacin are strongly bactericidal with activities approaching 1,000 times that of nalidixic acid (25). They are effective against a wider range of bacteria, including *Pseudomonas aeruginosa* and gram-positive cocci, which are considered resistant to nalidixic acid (12, 21). The activity of this family of drugs may be due to inhibition of bacterial DNA synthesis (25). Due to the great potential for the clinical use of these drugs it is important to investigate any possible positive or negative side effects on the host defense system.

In a previous study with human granulocytes no direct effects of the new quinolone derivatives were found, either on granulocyte chemotaxis, chemiluminescence, phagocytosis, or bacterial killing (15). However, uptake of tritiated thymidine into DNA was increased in human lymphocytes stimulated with phytohemagglutinin (PHA) when they were exposed to four of the new quinolone derivatives at clinically achievable concentrations (14). In contrast, no effect of ciprofloxacin and an inhibitory effect of ofloxacin and norfloxacin on [^{3}H]thymidine incorporation have been reported by two other laboratories (7, 17).

This study further examines the increased [^{3}H]thymidine incorporation in the presence of quinolones. The effect varied with time after lymphocyte stimulation. Moreover, ciprofloxacin inhibited lymphocyte growth, progression through the cell cycle, and immunoglobulin production, indicating that these drugs may also affect eucaryotic cell function in vitro.

* Corresponding author.

MATERIALS AND METHODS

Antimicrobial agents. Fresh solutions of the following preservative-free drugs were used: amifloxacin, ciprofloxacin, enoxacin, flumequine, pefloxacin, and pipemidic acid (Bayer, Wuppertal, Federal Republic of Germany), norfloxacin (Astra, Södertälje, Sweden), ofloxacin (Hoechst, Frankfurt, Federal Republic of Germany), A-56619 and A-56620 (Abbott Laboratories, North Chicago, Ill.), cinoxacin (Eli Lilly & Co., Indianapolis, Ind.), and nalidixic acid (Sterling Drug, New York, N.Y.).

Lymphocytes. Heparinized blood from healthy donors was used in most experiments. Mononuclear cells were harvested by centrifugation on Ficoll-Hypaque (3). The cells were then washed twice in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 5% fetal calf serum. Mononuclear cells enriched for B-lymphocytes were isolated by depletion of monocytes in plastic culture plates for 1 h at 37°C and removal of T-lymphocytes from the nonadherent cells by rosette formation with sheep erythrocytes treated with 2-aminoethylisothiouronium bromide with subsequent density gradient centrifugation (26). The Epstein-Barr virus-tranformed cell lines Raji, Laz 388, and Laz 509 and the human thymic tumor-derived cell line Rex were also used in some experiments.

Mitogens. T lymphocytes were activated with PHA (Welcome Ltd., London, England) at a final concentration of 1 μg/ml or concanavalin A (ConA) (25 μg/ml; Calbiochem-Behring, La Jolla, Calif.). Pokeweed mitogen in a final dilution of 1:200 was used to stimulate immunoglobulin secretion. B lymphocytes were activated with Formalin-

fixed *Staphylococcus aureus* Cowan strain 1 (0.05%, vol/vol, Pansorbin; Calbiochem-Behring) (16) or Epstein-Barr virus, obtained as the supernatant of cultures of the marmoset cell line B95-8 (26).

Lymphocyte culture conditions. Triplicate samples were cultured in 96-well microtiter plates containing 10^5 lymphocytes per culture in 200 μl of RPMI 1640 medium supplemented with 10% AB serum or 15% fetal calf serum, 2 mM glutamine, and 12 or 50 μg of gentamicin per ml. The effect of 4-quinolone drugs on human lymphocytes were identical in gentamicin-free medium. Mitogens giving maximal stimulation and 4-quinolone drugs were added at the beginning of the cultures unless otherwise stated. Cultures were incubated in 6% CO_2 at 37°C. The proliferation of lymphocytes and the cell line in the presence of ciprofloxacin was determined with an automatic cell counter (Coulter Electronics, Inc., Hialeah, Fla.).

Measurement of [^{3}H]thymidine incorporation. [^{3}H] Thymidine (1 μCi; NET-0272; specific activity, 78 Ci/mmol; New England Nuclear Corp, Boston, Mass.) was added to lymphocyte cultures to quantify DNA synthesis. Cultures were usually harvested in distilled water by an automatic harvesting machine onto glass fiber filters (Skatron A/S, Liberbyen, Norway). In some experiments, the filters were carefully washed with cold 10% trichloroacetic acid after harvesting. 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.

Cell cycle and cell volume analysis by flow cytometry. DNA content of individual cells stained with propidium iodide was analyzed by flow cytometry with a Coulter Epics C flowcytometer (5). Briefly, cells obtained from culture were washed once and stained with 50 μg of the DNA-specific fluorochrome propidium iodide (Sigma Chemical Co., St. Louis, Mo.) per ml in 0.1% sodium citrate buffer with 0.1% Triton X-100. The hypotonic solution caused cell lysis but left the stained nuclei intact. Between 10,000 and 20,000 nuclei per sample were analyzed. The amount of fluorescence was proportional to the DNA content of the cell, and fluorescence associated with each cell was calibrated by using propidium iodide-stained thymocytes as a standard. Data were collected in the form of DNA histograms of the cell populations in which the abscissa was the amplitude of fluorescence per cell (proportional to the quantity of DNA per cell) and the ordinate was the number of cells. The experimental data were computer processed. The size distribution of viable lymphocytes was approximated by the analysis of 90° light scatter properties with a Coulter Epics C.

Radioimmunoassays for immunoglobulin secretion. Solid-phase radioimmunoassays for immunoglobulin G (IgG) and IgM were performed in disposable flexible polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) (2). Plates were coated with either 100 μl of rabbit anti-human IgG or anti-human IgM (Dakopatts A/S, Denmark) overnight at room temperature. The plates were washed three times in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), incubated with 200 μl of 10% BSA–PBS for 1 h at room temperature, and washed once more with 1% BSA–PBS. Standard dilutions (100 μl) of normal human serum as well as supernatants of experimental samples diluted 50 times in 1% BSA–PBS were plated in triplicate. After overnight incubation the plates were washed three times, and 100 μl of 1% BSA–PBS containing 5 ng of ^{125}I-labeled rabbit anti-human IgG or anti-human IgM was added to each well. After a 5-h incubation at room temperature the plates were washed 10 times with PBS. Individual wells were cut apart and counted in an auto-gamma scintillation spectrometer (Packard). Standard curves in this radioimmunoassay were linear between 1 and 500 ng of immunoglobulin per ml. Whenever the concentration of immunoglobulin in a culture supernatant exceeded 500 ng/ml it was further diluted so it would fall into this range.

TABLE 1. [^{3}H]thymidine incorporation of PHA-stimulated human lymphocytes cultured for 5 days with different quinolone derivatives[a]

Antibiotic	[^{3}H]thymidine incorporation, % of control ± SEM at a drug concn (μg/ml) of:		
	25	6.25	1.56
Ciprofloxacin	331 ± 39[b]	237 ± 20[b]	152 ± 7[b]
Norfloxacin	294 ± 31[b]	162 ± 16[b]	111 ± 4[c]
Ofloxacin	296 ± 25[b]	182 ± 10[b]	132 ± 6[b]
A-56619	233 ± 30[b]	177 ± 18[b]	119 ± 8[c]
A-56620	263 ± 34[b]	143 ± 8[b]	106 ± 4
Amifloxacin	240 ± 20[b]	146 ± 7[b]	114 ± 4[c]
Enoxacin	218 ± 38[b]	214 ± 16[b]	130 ± 4[b]
Pefloxacin	298 ± 26[b]	183 ± 11[b]	136 ± 6[b]
Flumequine	177 ± 11[b]	121 ± 9	107 ± 8
Pipemidic acid	163 ± 12[b]	111 ± 6	103 ± 5

[a] Lymphocytes (10^5 per well) from eight different healthy donors were incubated with all antibiotics, and the results were statistically compared with control cultures without the drugs. The cells were incubated with [^{3}H]thymidine during the last 18 h of culture. The [^{3}H]thymidine uptake in control cultures without drugs was 100,000 to 140,000 cpm.

[b] There was a significant increase ($P < 0.01$) in [^{3}H]thymidine incorporation.

[c] There was a significant increase ($P < 0.05$) in [^{3}H]thymidine incorporation.

Statistics. The Wilcoxon matched-pair test was used to check the statistical significance of results.

RESULTS

Effects of 4-quinolone derivatives on thymidine incorporation in PHA-stimulated lymphocytes. The effects of 12 different 4-quinolones on [^{3}H]thymidine incorporation in PHA-stimulated human lymphocytes (T lymphocytes) were examined. Eight of the 4-quinolones at concentration of 1.56 to 6.25 μg/ml significantly increased [^{3}H]thymidine uptake of stimulated lymphocytes, with ciprofloxacin showing the strongest activity (Table 1). Washing with trichloroacetic acid did not influence these results. This suggests that the tritiated nucleoside is incorporated into trichloroacetic acid-precipitable material and does not represent a pool of unincorporated counts. Flumequine and pipemidic acid only had a significant effect at 25 μg/ml. Nalidixic acid and cinoxacin did not have an effect in the concentration range of 1.56 to 25 μg/ml.

To examine the effects of 4-quinolones over different exposure periods, lymphocytes were cultured in the presence or absence of ciprofloxacin, and [^{3}H]thymidine incorporation was assessed after 1 to 6 days of culture. Cultures harvested after 1 day (24 h) gave less than 5,000 cpm of [^{3}H]thymidine incorporation. After 2 days of culture with 1.25 to 5 μg of ciprofloxacin per ml, the [^{3}H]thymidine incorporation did not significantly differ from that in control cultures in five experiments (Fig. 1). However, at 20 μg of ciprofloxacin per ml, there was a significant ($P < 0.01$) decrease in incorporation. After 3 days of culture and on

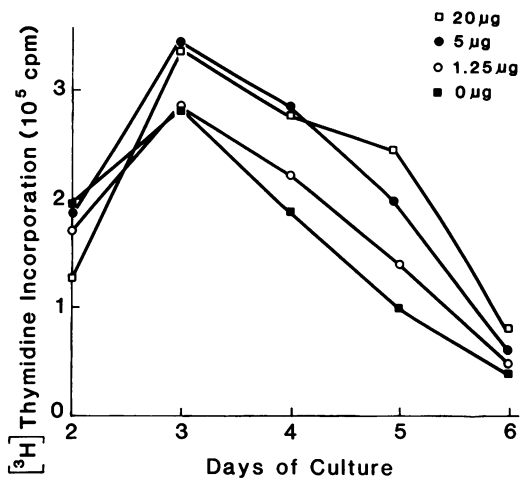

FIG. 1. [^{3}H]thymidine incorporation of PHA-stimulated human lymphocytes after 2 to 6 days in culture with different concentrations of ciprofloxacin. The cultures were pulsed with [^{3}H]thymidine 8 h before harvesting. These results are representative of those obtained in five experiments.

subsequent days the incorporation of [^{3}H]thymidine was increased in the presence of ciprofloxacin. In some experiments, a limited effect was seen on day 3 of culture, whereas the effect of ciprofloxacin on [^{3}H]thymidine incorporation was more obvious the following days. When the total radioactivity incorporated over the 6-day period (total area under the curve) was calculated from three different experiments, at 5 and 20 μg of ciprofloxacin per ml the [^{3}H]thymidine incorporation was increased by 42 to 45%. In contrast, at 80 μg of ciprofloxacin per ml, [^{3}H]thymidine incorporation was decreased by more than 90%. The effects of norfloxacin, ofloxacin, A-56619, and A-56620 on [^{3}H]thymidine incorporation was also significantly more pronounced after 5 days of culture than after 3 days (data not shown). PHA-stimulated lymphocytes were also pulsed with [^{3}H]thymidine 6, 8, 12,

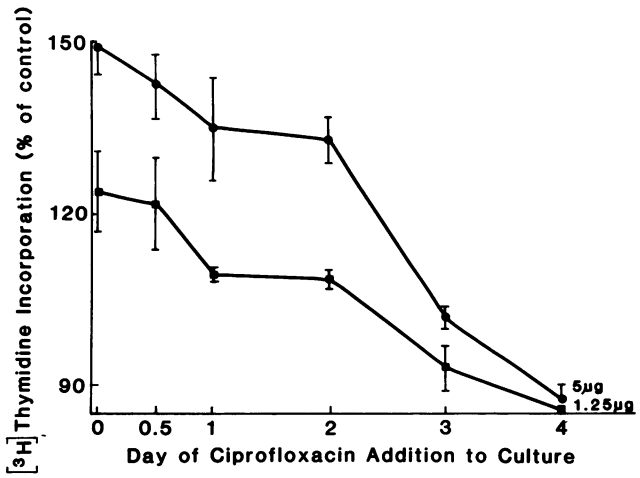

FIG. 2. Ciprofloxacin (5 and 1.25 μg/ml) was added to PHA-stimulated lymphocytes at various times during culture. Lymphocytes were pulsed with [^{3}H]thymidine during the last 16 to 18 h of culture and harvested on day 5. The results are expressed as percentage of control cultures that did not contain ciprofloxacin ± standard deviation.

TABLE 2. Ciprofloxacin pretreatment does not affect PHA-stimulated human lymphocytes

Ciprofloxacin concn (μg/ml)		[^{3}H]thymidine incorporation (cpm)[a]	% Increase
Preincubation (24 h)[b]	Incubation (120 h)		
0	0	239,897 ± 21,897	
5	0	242,321 ± 15,751	1
5	5	368,975 ± 19,515	54

[a] Values represent the mean counts per minute ± standard deviations of triplicate cultures.
[b] The lymphocytes were preincubated in the absence or presence of 5 μg of ciprofloxacin per ml and then washed twice with RPMI 1640.

and 18 h before harvesting on days 4 and 5 of culture. There was a similar increase in [^{3}H]thymidine uptake for all four pulsing times in ciprofloxacin-treated cultures (5 and 20 μg/ml) compared with control cultures.

Figure 2 demonstrates the effect of ciprofloxacin on [^{3}H]thymidine incorporation when added at the initiation and after 0.5, 1, 2, 3, and 4 days of culture to PHA-stimulated lymphocytes that were harvested at day 5. There was a strong stimulatory effect when ciprofloxacin was added during the first 24 h of culture and a moderate effect when ciprofloxacin was added after 2 days. Lymphocytes were also preincubated in the presence of 5 μg of ciprofloxacin per ml or in antibiotic-free medium for 24 h. The lymphocytes were then stimulated with PHA in the presence or absence of ciprofloxacin (5 μg/ml), and the effect on [^{3}H]thymidine incorporation was examined. Preincubation with ciprofloxacin for 24 h did not influence subsequent [^{3}H]thymidine incorporation after PHA stimulation (Table 2).

To determine whether ciprofloxacin also influenced thymidine incorporation of lymphocytes after stimulation with mitogens other than PHA, ConA (Table 3), pokeweed mitogen, and *S. aureus* Cowan strain 1 were added to human lymphocyte cultures containing 5 to 20 μg of ciprofloxacin per ml. During the peak of incorporation, which varied for the different mitogens, there was an enhanced uptake of [^{3}H]thymidine in a fashion similar to that for PHA-stimulated lymphocytes. When no mitogen was added to lymphocyte cultures a [^{3}H]thymidine uptake of approximately 1,000 cpm was recorded. Mean values of 1,600 cpm were recorded after 5 days of incubation with 5 or 20 μg of ciprofloxacin per ml added to these cultures.

Ciprofloxacin inhibits lymphocyte growth. PHA-stimulated human lymphocytes were cultured in the presence of different ciprofloxacin concentrations and harvested after 0 to 6 days, and cell multiplication was determined with a cell

TABLE 3. Ciprofloxacin increases the [^{3}H]thymidine incorporation of ConA-stimulated human lymphocytes[a]

Days of incubation	[^{3}H]thymidine uptake, cpm (% increase), at a ciprofloxacin concn (μg/ml) of:		
	0	5	20
3	16,430	16,716 (2)	20,465 (25)
4	33,073	44,001 (33)	54,887 (65)
5	43,938	64,924 (48)	75,218 (71)
6	29,889	44,091 (48)	88,077 (194)
7	24,793	29,133 (18)	37,097 (50)

[a] Human blood lymphocytes were stimulated with ConA and cultured with ciprofloxacin for 3 to 7 days. Cultures were pulsed for 16 h before harvesting. These results are representative of those obtained in three experiments and are means of triplicate cultures. Standard deviations were less than 15%.

TABLE 4. Ciprofloxacin inhibits an increase in cell number of PHA-stimulated lymphocytes[a]

Day of culture	No. of cells (10^6) at a ciprofloxacin concn (μg/ml) of:		
	0	5	20
0	0.29	0.30	0.31
4	0.90	0.70	0.54
5	2.04	1.94	1.37
6	2.39	2.08	1.31

[a] Blood lymphocytes were stimulated with PHA and incubated with ciprofloxacin for 1 to 6 days. Cultures were collected daily and counted in a Coulter counter. Results are means of triplicate cultures and are representative of results obtained in eight experiments.

counter. During the first 3 days of culture, no significant effect of ciprofloxacin was detected. However, after 4 or more days of culture the lymphocytes were shown to grow more slowly (approximately 50%) in the presence of 20 μg of ciprofloxacin per ml than in the control cultures (Table 4). The cell growth was insignificantly inhibited in cultures containing 5 μg of ciprofloxacin per ml.

When four different transformed human lymphoblastoid cell lines (Raji, Rex, LAZ 388, and LAZ 509) were incubated with ciprofloxacin, an influence on cell growth similar to that with PHA-stimulated lymphocytes was observed. After 3 days of culture, the cell number in control cultures had increased 8 times (to 8×10^5 cells per ml). However, cells incubated with 5 and 20 μg of ciprofloxacin per ml were inhibited in their growth by 0 to 30% and 50 to 67%, respectively.

Ciprofloxacin inhibits cell cycle progression. Although [^{3}H]thymidine incorporation is an estimate of DNA synthesis, it does not indicate the percentage of cells entering the cell cycle. By using a flow cytometry technique to quantitate the amount of DNA in individual cells, the percentage of cells in S and G_2/M phases of cycle was estimated (Fig. 3). Few cells cultured without PHA entered the G_2/M stage, whereas PHA activation induced a major portion to leave the G_0/G_1 stage, with the highest percentage of cells in S and G_2/M after 2 days of culture. Ciprofloxacin at the highest concentration, 20 μg/ml, inhibited the percentage of lymphocytes entering the S and G_2/M phases during the first 3 days of incubation. The highest percentage of cells in the S and G_2/M stages occurred on days 3 and 4 for cells treated with 5 ($P < 0.05$) and 20 ($P < 0.01$) μg of ciprofloxacin per ml, respectively. These results suggest that ciprofloxacin-treated cells progress more slowly through the S and G_2/M stages of the cell cycle, with a delayed return to G_1. Cultures containing 1.25 μg of ciprofloxacin per ml did not differ from control cultures. Similar results were obtained with lymphocytes stimulated by a different mitogen, ConA (data not shown).

Increases in cell size after PHA activation occur before and are a prerequisite for the initiation of DNA synthesis (20). Lymphocytes cultured with 5 μg of ciprofloxacin per ml did not differ from control cells when Wright-Giemsa-stained smears were examined by microscopy. However, light microscopy examination of cells exposed to 20 μg of ciprofloxacin per ml showed maximum enlargement on day 2 to 3 after PHA stimulation, approximately 1 day later than the control cells not exposed to ciprofloxacin. Microscopic examination of lymphocytes exposed to 80 μg of ciprofloxacin per ml revealed resting lymphocytes on days 2 to 3 after PHA activation with only some detectable enlargement on day 4 or 5. In addition, the size distribution of cells, approximated by 90° light scatter properties, showed that cell size did not decrease at day 4 and 5 for cells treated with 20 μg of ciprofloxacin per ml, whereas it did decrease for cells not treated with the drug.

Ciprofloxacin inhibits immunoglobulin production. Lymphocytes (B and T cells) were stimulated with pokeweed mitogen and immunoglobulin secretion into the culture medium assessed. Supernatant fluid from triplicate cultures was harvested daily and analyzed for IgG and IgM content (Fig. 4). The secretion of IgG and IgM was dramatically inhibited by 20 μg of ciprofloxacin per ml and decreased to approximately half at 5 μg/ml, whereas 1.25 μg of ciprofloxacin per ml had a limited influence on immunoglobulin production. In addition, when isolated human B lymphocytes were stimulated with Epstein-Barr virus the IgG and IgM production was inhibited to the same degree.

DISCUSSION

Most antibacterial drugs do not affect the mitogenic response of human T and B lymphocytes, whereas a few are inhibitory (1, 11, 13). Therefore, the finding of a pronounced alteration in the incorporation of tritiated thymidine in the presence of 4-quinolones was remarkable (Tables 1 and 2; Fig. 1). The results are even more interesting in view of the fact that with some of the new quinolones the effect is even apparent at clinically achievable concentrations (Table 1). For ciprofloxacin, a mean peak level in serum of 1.30 μg/ml with a range from 0.15 to 2.5 μg/ml after an oral dose of 500 mg was reported for elderly men (18). The corresponding mean peak level in tissue (prostate) was 3.03 μg/ml with a range up to 5 μg/ml (18). The peak level in urine after 500 mg of ciprofloxacin has been reported to be 300 to 400 μg/ml (6).

The increased thymidine incorporation by quinolones into lymphocytes suggests a true stimulation of DNA synthesis. However, lymphocyte growth was inhibited at 20 μg of ciprofloxacin per ml, and cell cycle analysis indicated a delay in DNA synthesis and an inhibited progression through the cell cycle at 5 and 20 μg/ml. Thus, the increased [^{3}H]thymidine incorporated is not reflecting an increase in normal DNA replication and cell growth. In addition, B-cell immunoglobulin secretion in T cell-dependent and -independent assays was inhibited, suggesting that lymphocyte function in vitro is altered by the presence of ciprofloxacin.

There is a discrepancy between our results showing an

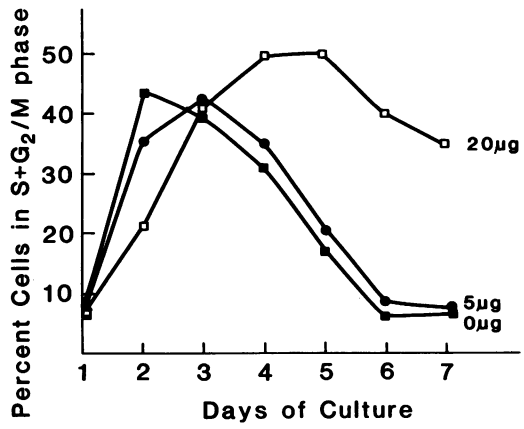

FIG. 3. Ciprofloxacin increases the percentage of lymphocytes in the S and G_2/M phases of the cell cycle. Lymphocytes were stimulated with PHA and grown in the presence or absence of ciprofloxacin. DNA content of individual cells stained with propidium iodide was analyzed by flow cytometry.

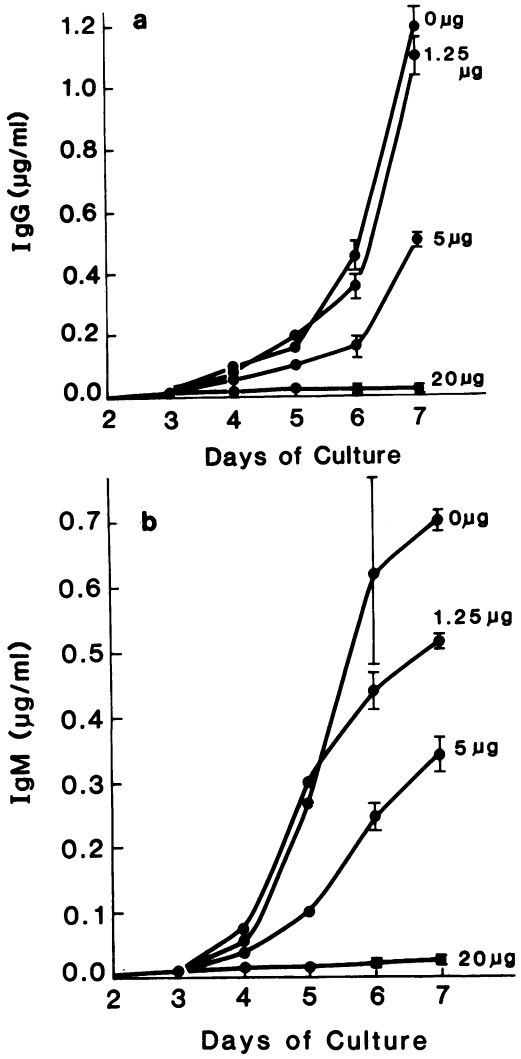

FIG. 4. Secretion of IgG and IgM by pokeweed mitogen-stimulated lymphocytes in the presence of ciprofloxacin. Lymphocytes were cultured in microtiter plates, and supernatant fluid was harvested daily. The immunoglobulin content in the supernatants was determined with a radioimmunoassay. These results are representative of those obtained in five experiments.

increased [^{3}H]thymidine uptake and those of De Simone et al. (7) and Gollapudi et al. (17) demonstrating an inhibitory or no effect in human lymphocyte cultures stimulated with PHA or ConA for 3 days. In our kinetic experiments there was no increase in [^{3}H]thymidine uptake at day 2 in drug-treated cultures compared with control cultures. However, an increased [^{3}H]thymidine uptake by the drug-treated cells was significant at day 3 and even more pronounced on the subsequent days. If the cells cultured by De Simone et al. and Gollapudi et al. grew slightly slower or were harvested somewhat earlier than our 3-day cultures, the effect of quinolones on [^{3}H]thymidine incorporation would not have been detected.

Ciprofloxacin, one of the most active of the new 4-quinolones, penetrates leukocytes effectively (27). This suggests that the new quinolones may have the capacity to inhibit intracellular events in lymphocytes. The present study does not reveal the molecular target(s) for quinolones in lymphocytes. In an unconfirmed study, nalidixic acid at very high concentrations has been found to inhibit topoisomerases of HeLa cells (22). In addition, DNA polymerases α and β and terminal deoxynucleotidyl transferase are inhibited by quinolone antibiotics (nalidixic acid, oxolinic acid, and pefloxacin) (23). A significant decrease in the amount of synthesized DNA in mammalian cells was only observed with concentrations of more than 100 μg of the new 4-quinolones and DNA polymerase α; topoisomerases I and II were also only inhibited at high concentrations (19). DNA topoisomerase II from eucaryotic cell nuclei has a different molecular conformation than bacterial topoisomerase II (4, 22, 25). Thus, neither the increased [^{3}H]thymidine uptake nor the growth inhibition can be explained by inhibition of topoisomerase II. However, an interesting aspect of the results presented here is that the capacity of the quinolones to induce uptake of [^{3}H]thymidine correlates with their antibacterial effect in *E. coli* (12, 21).

The target responsible for the killing effect of the quinolones in bacteria has been suggested to be DNA topoisomerase II (DNA gyrase) (24; for a review see reference 9). An unconfirmed study indicates that the mechanism of inhibition of bacterial DNA topoisomerase II by quinolones is indirectly through DNA binding (25). Recently in a study on the effect of 4-quinolones on topoisomerase II from *Micrococcus luteus* it was found that the effect did not always correlate with antimicrobial potency (28). Instead, the results suggested that other factors in addition to inhibition of gyrase supercoiling activity are of importance for the potency of these drugs.

Novobiocin is an inhibitor of eucaryotic class II topoisomerases and DNA polymerase α and also an inhibitor of mitochondria resulting in a decrease in the cellular ATP/ADP ratio (8). Mitochondria contain a distinct DNA topoisomerase different from the nuclear topoisomerase (10). Therefore, the new 4-quinolones may inhibit mitochondrial function, resulting in an inhibited progression through the cell cycle, especially during the most energy requiring stages of S and G_2/M. Other unexplored possibilities for the action of 4-quinolones on lymphocytes and possibly also on bacteria is through DNA damage requiring repair or giving gene amplification, thereby requiring increased [^{3}H]thymidine incorporation. Inhibition of pyrimidine biosynthesis could result in increased [^{3}H]thymidine incorporation.

The present report confirms that the new 4-quinolones increase the incorporation of [^{3}H]thymidine in human lymphocytes (14). Ciprofloxacin, one of the antibacterially most active 4-quinolones, was shown to inhibit lymphocyte growth, progression through the cell cycle, and immunoglobulin secretion in vitro. Since some of these effects were apparent at clinically achievable concentrations, the possible effects of the new 4-quinolones on in vitro and in vivo eucaryotic cell function warrant additional study.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants CA25369 and CA34183 from the National Institutes of Health and by the Swedish Medical Research Council. T.F.T. is a Research Fellow (grant DRG-864) of the Damon Runyon-Walter Winchell Cancer Fund.

We thank our colleagues Ann Penta for help with these experiments, Herbert Levine for flow cytometry analysis of cells, and Arthur Pardee and Victor Goldmacher for helpful suggestions.

LITERATURE CITED

1. **Banck, G., and A. Forsgren.** 1979. Antibiotics and suppression of lymphocyte function in vitro. Antimicrob. Agents Chemo-

ther. **16**:554–560.
2. **Bensussan, A., S. C. Meuer, S. F. Schlossman, and E. L. Reinherz.** 1984. Delineation of an immunoregulatory amplifier population recognizing autologous Ia molecules. J. Exp. Med. **159**:559–576.
3. **Boyum, A.** 1968. Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. **21** (Suppl. 97):9.
4. **Chen, G. L., L. Yang, T. C. Rowe, B. D. Halligan, K. M. Tewey, and L. F. Liu.** 1984. Nonintercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J. Biol. Chem. **259**:13560–13566.
5. **Crissman, H. A., and J. A. Steinkamp.** 1973. Rapid simultaneous measurement of DNA, protein, and cell volume in single cells from large mammalian cell populations. J. Cell Biol. **59**:766–771.
6. **Davis, R. L., J. R. Koup, J. Williams-Warren, A. Weber, and A. L. Smith.** 1985. Pharmacokinetics of three oral formulations of ciprofloxacin. Antimicrob. Agents Chemother. **28**:74–77.
7. **De Simone, C., L. Baldinelli, M. Ferrazzi, S. DeSantis, L. Pugnaloni, and F. Sorice.** 1986. Influence of ofloxacin, norfloxacin, nalidixic acid, pyromidic acid and pipemidic acid on human γ-interferon production and blastogenesis. J. Antimicrob. Chemother. **17**:811–814.
8. **Downes, S. C., M. J. Ord, A. M. Mullinger, A. R. S. Collins, and R. T. Johnson.** 1985. Novobiocin inhibition of DNA excision repair may occur through effects on mitochondrial structure and ATP metabolism, not on repair topoisomerases. Carcinogenesis **6**:1343–1352.
9. **Drlica, K.** 1984. Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. **48**:273–289.
10. **Fairfield, F. R., W. R. Bauer, and M. V. Simpson.** 1979. Mitochondria contain a distinct DNA topoisomerase. J. Biol. Chem. **254**:9352–9354.
11. **Forsgren, A.** 1981. The immunosuppressive effect of cephalosporins and cephalosporin-gentamicin combinations. J. Antimicrob. Chemother. **8**(Suppl. B):183–186.
12. **Forsgren, A.** 1985. Comparative *in vitro* activity of three new quinolone antibiotics against recent clinical isolates. Scand. J. Infect. Dis. **17**:91–94.
13. **Forsgren, A., and G. Banck.** 1978. Influence of antibiotics on lymphocyte function *in vitro*. Infection **6**(Suppl. 1):91–97.
14. **Forsgren, A., A.-K. Bergh, M. Brandt, and G. Hansson.** 1986. Quinolones affect thymidine incorporation into the DNA of human lymphocytes. Antimicrob. Agents Chemother. **29**: 506–508.
15. **Forsgren, A., and P. I. Bergkvist.** 1985. Effect of ciprofloxacin on phagocytosis. Eur. J. Clin. Microbiol. **4**:575–578.
16. **Forsgren, A., A. Svedjelund, and H. Wigzell.** 1976. Lymphocyte stimulation by protein A of *Staphylococcus aureus*. Eur. J. Immunol. **6**:207–213.
17. **Gollapudi, S. V. S., R. H. Prabhala, and H. Thadepalli.** 1986. Effect of ciprofloxacin on mitogen-stimulated lymphocyte proliferation. Antimicrob. Agents Chemother. **29**:337–338.
18. **Grabe, M., A. Forsgren, and T. Björk.** 1986. Concentrations of ciprofloxacin in serum and prostatic tissue in patients undergoing transurethral resection. Eur. J. Clin. Microbiol. **5**:211–212.
19. **Hussy, P., G. Maas, B. Tümmler, F. Grosse, and U. Schomburg.** 1986. Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase primase complex, topoisomerases I and II and growth of mammalian lymphoblasts. Antimicrob. Agents Chemother. **29**:1073–1078.
20. **Kehrl, J. H., A. Muraguchi, and A. S. Fauci.** 1984. Human B cell activation and cell cycle progression: stimulation with anti-μ and *Staphylococcus aureus* Cowan strain I. Eur. J. Immunol. **14**:115–121.
21. **King, A., K. Shannon, and J. Philips.** 1984. The *in vitro* activity of ciprofloxacin compared with that of norfloxacin and nalidixic acid. J. Antimicrob. Chemother. **13**:325–331.
22. **Miller, K. G., L. F. Liu, and P. T. Englund.** Homogenous type II DNA topoisomerase from HeLa cell nuclei. J. Biol. Chem. **256**:9334–9339.
23. **Rusquet, R., M. Bonhommet, and J. C. David.** 1984. Quinolone antibiotics inhibit eucaryotic DNA polymerase alpha and beta terminal deoxynucleotidyl transferase but not DNA ligase. Biochem. Biophys. Res. Commun. **121**:762–769.
24. **Shen, I. I., and A. G. Pernet.** 1985. Mechanism of inhibition of DNA gyrase by analogues of nalidixic acid: the target of the drugs is DNA. Proc. Natl. Acad. Sci. USA **82**:307–311.
25. **Smith, J. T.** 1984. Awakening the slumbering potential of the 4-quinolone antibacterials. Pharmaceut. J. **233**:209–305.
26. **Tedder, T. F., A. W. Boyd, A. S. Freedman, L. M. Nadler, and S. F. Schlossman.** 1985. The B cell surface molecule B1 is functionally linked with B cell activation and differentiation. J. Immunol. **135**:973–979.
27. **Traub, W. H.** 1984. Intraphagocytic bactericidal activity of bacterial DNA gyrase inhibitors against *Serratia marcescens*. Chemotherapy **30**:379–386.
28. **Zweerink, M. M., and A. Edison.** 1986. Inhibition of *Micrococcus luteus* DNA gyrase by norfloxacin and 10 other quinolone carboxylic acids. Antimicrob. Agents Chemother. **29**:598–601.