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Fluorinated 4-quinolones induce hyperproduction of interleukin 2

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ABSTRACT **The fluorinated 4-quinolones are a "new" group of antibiotics with a broad antibacterial spectrum. They are already widely used in clinical practice. Previous studies have shown that these drugs increase the uptake of [^{3}H]-thymidine into DNA of mitogen-stimulated lymphocytes but inhibit cell growth and immunoglobulin secretion. This study shows that the 4-quinolones strongly (up to 100 times) increase the recovery of interleukin 2 (IL-2) in culture supernatants of phytohemagglutinin (PHA)-stimulated normal human lymphocytes and also prolong the kinetics of IL-2 production. The effect was significant at clinically achievable concentrations (5 μg/ml). In addition to hyperproduction of IL-2, the level of RNA hybridizing with a human IL-2 cDNA probe was also intensely elevated (16–32 times) in PHA-stimulated lymphocytes cultured with ciprofloxacin (80 μg/ml). The mechanism responsible for 4-quinolone-mediated effects on T cells is at present unclear, but evidence is presented that suggests the effect is not exerted at the level of protein kinase C activation. Ciprofloxacin at 80 μg/ml also decreased the expression of IL-2 receptors measured by immunofluorescence with CD 25 antibodies and a radiolabeled IL-2 binding assay. At the same concentration of ciprofloxacin, there was a very low expression of the transferrin receptor and the cell size increased very little in human lymphocytes after PHA stimulation. The enhanced IL-2 production by 4-quinolones may contribute to side effects reported when these drugs are used for treatment of patients.**

The "new" 4-quinolones are strongly bactericidal and have a very broad antibacterial spectrum. They are fluorinated predecessors to nalidixic acid and the antibacterial activity is due to inhibition of DNA synthesis resulting from inhibition of DNA gyrase activity (1). Ciprofloxacin, enoxacin, norfloxacin, ofloxacin, and pefloxacin have been most extensively studied but many other 4-quinolones have been synthesized and are under development. Because the 4-quinolones have proven to be highly effective in most clinical trials, they are already widely used in clinical practice. In general, the drugs appear to be safe and well tolerated (2).

In previous studies, we have shown that the new 4-quinolones significantly increase the uptake of [^{3}H]thymidine into DNA of mitogen-stimulated lymphocytes. In addition, ciprofloxacin, one of the most powerful 4-quinolones, which was investigated in more detail, inhibited cell growth, cell-cycle progression, and immunoglobulin secretion (3, 4).

T lymphocytes can be activated by a variety of stimuli, including specific monoclonal antibodies and mitogenic lectins. The appropriate stimulation of T lymphocytes has been shown to induce the production of the growth factor interleukin 2 (IL-2), as well as specific receptors for IL-2 (5, 6), and it is the subsequent interaction between IL-2 and its receptor that leads to initiation of DNA synthesis and proliferation (7–10). The functional receptor for IL-2 is composed of two noncovalently linked 55-kDa (Tac antigen; CD 25) and 75-kDa polypeptide chains (11–13). IL-2 is a lymphokine that has attracted much attention during recent years and, considering our previous studies on the effects of new 4-quinolones on T lymphocytes (3, 4), it was of interest to evaluate the effects of these antibiotics on the IL-2 and IL-2 receptor system. Quite unexpectedly, 4-quinolones have a dramatic synergistic effect on phytohemagglutinin (PHA)-induced IL-2 production resulting in hyperproduction of IL-2, which was demonstrated both at the level of IL-2 activity in culture supernatants as well as induction and accumulation of specific mRNA for IL-2. In contrast, however, the effect of 4-quinolones on the IL-2 receptor expression was less pronounced.

MATERIALS AND METHODS

Antimicrobial Agents. Fresh solutions of the following preservative-free drugs were used: amifloxacin and ciprofloxacin (Bayer, Wuppertal, F.R.G.), enoxacin (Warner–Lambert, Ann Arbor, MI), norfloxacin (Astra, Södertälje, Sweden), ofloxacin (Hoechst, Frankfurt), pefloxacin (Rhône Poulenc, Antom, France), nalidixic acid (Sterling, New York), bensylpenicillin (Astra), and gentamicin (Schering, Kenilworth, NJ).

Lymphocytes and Culture Conditions. Human peripheral lymphocytes (PBLs) were obtained from heparinized blood from healthy donors by centrifugation on a step gradient of mixed Isopaque (1 part) and Macrodex (2 parts), followed by separation on Ficoll-Isopaque (Lymphoprep; Pharmacia). Lymphocytes were cultured at a density of 1×10^6 cells per ml in RPMI 1640 medium supplemented with 10% fetal calf serum and 12 μg of gentamicin per ml in a humidified atmosphere of 5% CO_2/95% air. PHA (Wellcome) was used at a final concentration of 1 μg/ml.

IL-2 Biological Activity. IL-2 activity in lymphocyte culture supernatants was determined by the IL-2 concentration-dependent stimulation of proliferation of a cloned cytolytic T-lymphocyte line CTLL-2 (14). CTLL proliferation was monitored by [^{3}H]thymidine incorporation. [^{3}H]Thymidine (1 μCi/ml; 85.6 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) was added during the last 4 hr of a 24-hr culture period in the presence of serial 1:2 dilutions of the experimental sample and a standard IL-2 preparation calibrated to an international IL-2 standard preparation (Biological Response Modifiers Program). The IL-2 concentration (units/ml) in the experimental samples was obtained by comparison of regression lines for experimental samples and standard preparation.

Immunofluorescence Staining and Flow Cytometer Analysis. Fluorescein-conjugated anti-IL-2 receptor monoclonal antibodies (CD 25) (Becton Dickinson), antibodies to the transferrin receptor (OKT 9) (Ortho Diagnostics), and fluorescein-

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Abbreviations: IL-2, interleukin 2; PHA, phytohemagglutinin; PBL, peripheral lymphocyte; CTLL, cytolytic T lymphocyte; PDB, phorbol dibutyrate; PMA, phorbol 12-myristate 13-acetate.

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conjugated rabbit anti-mouse antibodies (Dakopatts, Glostrup, Denmark) were used according to the manufacturers' instructions.

A FacScan flow cytometer (Becton Dickinson) in which 10,000 events from every sample were registered and stored in the computer was used. The autofluorescence was determined with nonconjugated lymphocytes. By setting a gate, PBLs with a lower grade of viability as revealed by propidium iodine were not registered.

RNA Isolation, Blotting, and Autoradiography. Total cellular RNA was prepared from 200 × 10^6 cells by the guanidinium isothiocyanate/cesium chloride method (15). A Minifold I apparatus (Schleicher & Schüll, Dassel, F.R.G.) was used to blot the RNA onto nitrocellulose paper (Schleicher & Schüll membrane filter; 0.45 μm) as described by the manufacturer. The samples were diluted serially 1:2 and the first dilution represented 10 μg of total RNA. For electrophoretic blot hybridization, 5 μg of total RNA was loaded onto formaldehyde-agarose gels after denaturation at +65°C in formamide (16) and subsequently blotted to nitrocellulose. All filters were hybridized at +65°C in a mixture containing 5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate), 5× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 0.1% SDS, and 0.05 M EDTA for 14–16 hr. Final stringency in the washings was 0.1× SSC/0.1% SDS, 60°C, after which the filters were exposed to x-ray film for 24 hr at −70°C with an intensifying screen.

DNA Probes. A probe containing the entire coding region for human IL-2 was constructed from a partial cDNA obtained from P. Peterson (University of Uppsala) to which synthetic oligonucleotides were added. Upon screening, the synthetic cDNA displayed the correct IL-2 sequence as published by Taniguchi *et al.* (17). The cDNA probe specific for β-actin (PAL 41) was a kind gift from M. Buchingham (Pasteur Institute, Paris) to T. Leanderson in our laboratory. Probes were labeled with [^{32}P]dCTP (specific activity, 3000 Ci/mmol) by nick-translation (16) and the free nucleotides were separated by passage over a Sephadex G-50 fine column.

Radiolabeled IL-2 Binding Assay. Preparation of biosynthetically radiolabeled [^{3}H]IL-2 and radiolabeled IL-2 binding to intact cells was performed as described (18). All cells were prepared for the assay by centrifugation, followed by incubation at 37°C in IL-2-free RPMI 1640 medium for 4 hr to facilitate dissociation and/or degradation of endogenously bound IL-2. The calculated values of the number of binding sites per cell were obtained by Scatchard analysis of equilibrium binding data after subtraction of the nonspecific binding determined in the presence of 150-fold molar excess of unlabeled IL-2. The lower detection limit of receptor sites per cell was 50.

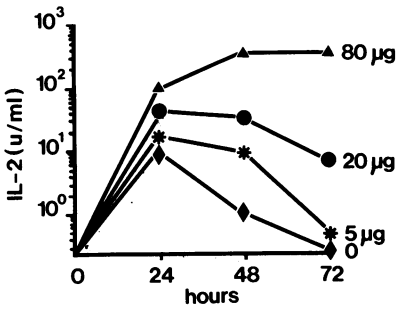

FIG. 1. Kinetics of IL-2 production by PHA-activated normal human lymphocytes (10^6 cells per ml) cultured with ciprofloxacin in concentrations of 80 (▲), 20 (●), and 5 (*) μg/ml, and without drug (◆). IL-2 activity in culture supernatants was measured by a CTLL assay. Mean values for experiments with lymphocytes from four different donors are indicated.

Table 1. Peak IL-2 activity in culture supernatants of PHA-stimulated human lymphocytes cultured with 4-quinolones

Drug	Drug concentration, μg/ml		
	80	20	5
Amifloxacin	35 ± 10 (1)	39 ± 11 (1)	16 ± 4 (1)
Ciprofloxacin	617 ± 204 (2)	53 ± 16 (2)	17 ± 3 (1)
Enoxacin	712 ± 218 (2)	148 ± 70 (2)	15 ± 2 (1)
Norfloxacin	58 ± 22 (2)	89 ± 40 (1)	21 ± 5 (1)
Ofloxacin	101 ± 42 (2)	27 ± 7 (1)	14 ± 2 (1)
Pefloxacin	235 ± 41 (2)	48 ± 11 (1)	16 ± 2 (1)
Nalidixic acid	11 ± 2 (1)	12 ± 2 (1)	10 ± 1 (1)
	No drug	9 ± 2 (1)	

Normal human lymphocytes (10^6 cells per ml) were cultured with PHA (1 μg/ml) in RPMI 1640 medium with the addition of 4-quinolones at different concentrations. The values demonstrate peak IL-2 activity during 24–72 hr of incubation and are the mean values ± SEM of experiments with lymphocytes from six healthy donors for each concentration and drug. The values for all drugs and concentrations except nalidixic acid were significantly different from the controls ($P < 0.05$). Numbers in parentheses indicate day of harvest.

^{32}P Labeling of Cells and Analysis of the 80-kDa Phosphoprotein. Cells were prelabeled with carrier-free $^{32}P_i$ for 6 hr as described (19). Cells were thereafter treated with ciprofloxacin in the presence or absence of mitogenic stimulation with anti-CD3 or the phorbol ester phorbol dibutyrate (PDB) and subsequently lysed in lysis buffer (100 mM NaH_2PO_4/5 mM phenylmethylsulfonyl fluoride/10 mM EDTA/5 mM EGTA/10 mM NaF/20 mM sodium pyrophosphate/1% Triton X-100/0.15 M NaCl, pH 7.5). Enrichment for the 80-kDa phosphoprotein was performed on the cleared lysate as described (20). The resulting phosphoproteins were analyzed by SDS/PAGE on an 11% gel under reducing conditions (2). To improve the resolution of the 80-kDa protein, gels were subjected to alkali treatment (20, 21). Thereafter, gels were dried down onto paper for autoradiography.

Statistics. Student's *t* test for paired data was used to calculate the statistical significance of differences.

RESULTS

4-Quinolones Induce Hyperproduction of IL-2. The characteristic kinetics of IL-2 accumulation in culture supernatants of normal human lymphocytes stimulated with PHA is shown in Fig. 1. In cultures not exposed to 4-quinolones, there was a transient peak at 24 hr of culture of maximally 10 units of IL-2 per ml of supernatant. After 48 and 72 hr of culture, very low levels of IL-2 could be detected in the culture medium. Identical kinetics and level of IL-2 production were also obtained in cultures containing 5–80 μg of the "old" non-

Table 2. IL-2 activity in Jurkat cells incubated with substimulatory concentrations of PMA and UCHT-1 antibodies (anti-T3) with and without ciprofloxacin

Ciprofloxacin, μg/ml	PMA, μg/ml			
	0	1.25	5	20
0	<0.25	0.5	0.5	0.5
20	<0.25	5	8.5	11
80	<0.25	6.5	11	17

The T-cell line Jurkat was cultured at a concentration of 1 × 10^6 cells per ml in RPMI 1640 medium in the presence of UCTH-1 antibodies at 4 μg/ml and the indicated concentration of PMA; ciprofloxacin IL-2 activity in the supernatants was determined after 18 hr of culture. Results are expressed as IL-2 units per ml of culture supernatant as determined by CTLL assay. Lower limit of the assay was 0.25 unit/ml.

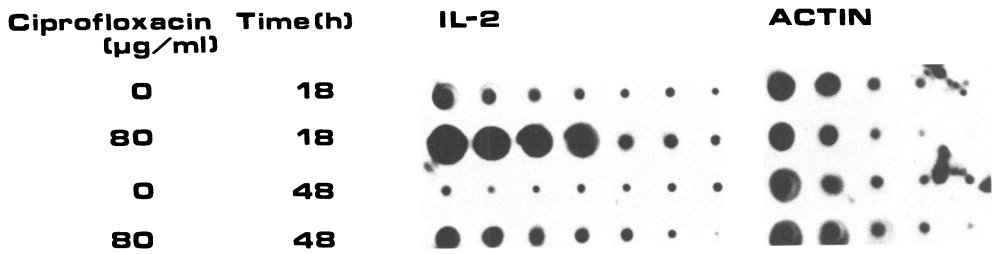

FIG. 2. Ciprofloxacin induces hyperinduction of IL-2 mRNA. Autoradiogram of dot blotting of cDNA (IL-2 and actin) with 1:2 dilutions of mRNA for IL-2 and actin from PHA-stimulated human lymphocytes grown at a density of 1×10^6 cells per ml for 18 and 48 hr in the presence or absence of ciprofloxacin added to cultures at the onset.

fluorinated 4-quinolone nalidixic acid, gentamicin, or benzylpenicillin per ml (data not shown).

However, ciprofloxacin, one of the new fluorinated 4-quinolones, caused a dose-dependent increase in the recovery of IL-2 in the culture supernatants. The levels were strongly increased after only 24 hr, and accumulated IL-2 activity continued to increase until 72 hr of culture at the highest antibiotic concentration (80 μg/ml). The peak levels of IL-2 in the presence of 80 μg of ciprofloxacin per ml were up to 100 times higher than the IL-2 levels in the antibiotic-free controls. The effect of lower concentrations (5–20 μg/ml) of ciprofloxacin was more modest with declining IL-2 levels after 48 hr.

Similar kinetics of increased IL-2 concentration in culture supernatants was also demonstrated when PHA-stimulated lymphocytes were incubated with new 4-quinolones other than ciprofloxacin. The highest levels were obtained with enoxacin and pefloxacin. Table 1 summarizes the peak concentrations of IL-2 activity obtained during 72 hr of incubation with 5, 20, and 80 μg of the different 4-quinolones per ml. As shown, there were also increased IL-2 levels with all new 4-quinolones at 5 μg/ml, a concentration that is achievable in serum in patients after oral administration of the new 4-quinolones. In cultures containing the new 4-quinolones in the absence of PHA, IL-2 could not be recovered in the supernatants (data not shown). Control experiments showed that the 4-quinolones did not by themselves influence the CTLL assay of concentrations used for IL-2 determinations.

To study IL-2 production in a clonal T-cell line in the absence of monocytes, the T-cell leukemia cell line Jurkat was used. This cell line produces IL-2 when stimulated with monoclonal antibodies to the T3 antigen (anti-CD3) in the presence of a phorbol ester—i.e., PDB or phorbol 12-myristate 13-acetate (PMA) (22). When Jurkat cells were exposed to suboptimal concentrations of these stimuli, an increased (0.5 unit/ml) IL-2 production occurred, but addition of ciprofloxacin caused a strong enhancement of IL-2 production (5–17 units/ml) (Table 2). However, ciprofloxacin could not in the absence of the other stimuli cause IL-2 production in Jurkat cells (data not shown). Ciprofloxacin thus seems to have a direct effect on the responding T-cell population and cannot replace the action of phorbol esters.

Ciprofloxacin Increases Specifically the Level of IL-2 mRNA in PHA-Stimulated Lymphocytes. The data presented above show increased levels of IL-2 in supernatants of activated lymphocytes incubated with the new 4-quinolones. To provide further evidence of an increased IL-2 production, the level of IL-2 mRNA was measured. In human lymphocytes, the concentration of RNA hybridizing to a human IL-2 cDNA was intensely elevated (16–32 times) in cells stimulated with PHA and cultured with ciprofloxacin (80 μg/ml) as revealed by dot blot hybridization (Fig. 2). As also shown, the level of actin mRNA was not affected by ciprofloxacin. Electrophoretic blot hybridization analysis revealed that the increase in hybridization to the IL-2 probe was due to the selective increase in one band corresponding to the native 1.2-kilobase IL-2 mRNA (data not shown).

Ciprofloxacin Does Not Activate Protein Kinase C. The results outlined above suggest that ciprofloxacin has a similar synergistic augmenting effect as phorbol esters on IL-2 production (23, 24). It has been shown that the main effect of phorbol esters is due to binding to and activation of protein kinase C (25, 26). To evaluate a possible ciprofloxacin specific effect on this kinase, we have analyzed the phosphorylation level at an 80-kDa intracellular protein. This phosphoprotein has been widely used as a marker for the activation of protein kinase C in various cellular systems (20, 21, 27–29). Accordingly, ^{32}P-labeled intact T lymphocytes were treated with various concentrations of ciprofloxacin and thereafter stimulated with either an anti-CD3 antibody or a phorbol ester. As shown in Fig. 3, both anti-CD3 and phorbol ester induced a marked phosphorylation of an 80-kDa cellular protein, while ciprofloxacin was ineffective at all concentrations tested. Moreover, it is also evident from the same figure that this drug does not synergize or antagonize with anti-CD3 or phorbol ester on the level of phosphorylation of the 80-kDa protein. These results suggest that the observed synergistic effect of ciprofloxacin on the level of IL-2 production is not due to protein kinase C activation.

Ciprofloxacin Influences Cell Size, IL-2 Receptors, and Transferrin Receptors. Ciprofloxacin has been shown to inhibit cell-cycle progression and function of human lymphocytes (3, 4). As the present report demonstrates a hyperinduction of IL-2, it was of interest also to investigate cell size, IL-2 receptors, and transferrin receptors of human lymphocytes stimulated with PHA and cultured with ciprofloxacin. Fig. 4*A* shows the cell size of PHA-stimulated lymphocytes. There is a considerable increase in cell size at 72 hr for cells not exposed to ciprofloxacin and those exposed to 20 μg of ciprofloxacin per ml. In contrast, the size of PHA-stimulated lymphocytes exposed to 80 μg of ciprofloxacin per ml does not increase during 72 hr of incubation. Fig. 4 *B* and *C* demonstrates the expression of the transferrin receptor (Fig. 4*B*) and the Tac antigen (Fig. 4*C*) in PHA-stimulated lymphocytes after 72 hr of culture with or without ciprofloxacin.

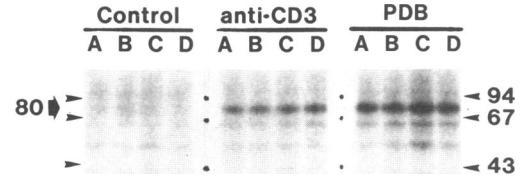

FIG. 3. Human T-cell blasts prelabeled with $^{32}P_i$ were exposed to graded concentrations of ciprofloxacin for 10 min (lanes: A, 0 μg/ml; B, 5 μg/ml; C, 20 μg/ml; D, 80 μg/ml). Cells were thereafter either left untreated or subsequently stimulated for 30 min with anti-CD3 (UCHT-1, 4 μg/ml) and cross-linking rabbit anti-mouse IgG1 (8 μg/ml) or PDB (20 μg/ml) as indicated. ^{32}P-labeled 80-kDa substrate (arrow) was visualized by autoradiography of the corresponding gel. Molecular masses are given in kDa.

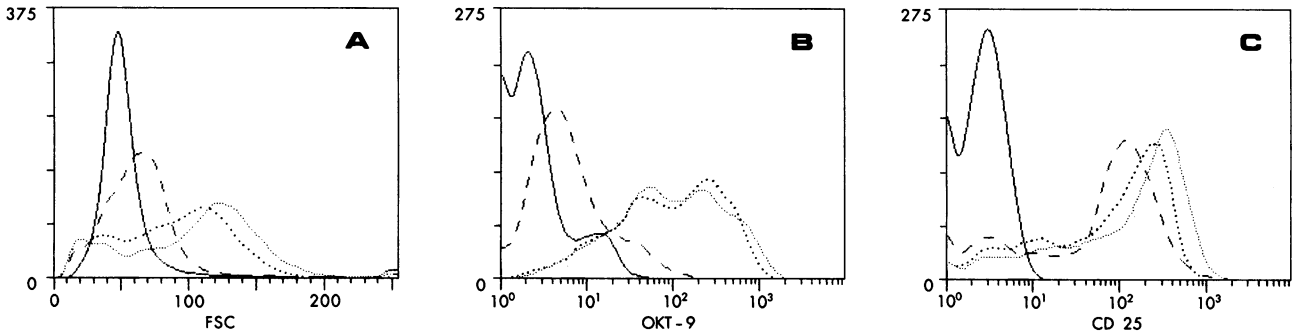

FIG. 4. Flow cytometer analysis of cell size (*A*), transferrin receptors (*B*), and IL-2 receptors (*C*) of human PHA-stimulated lymphocytes incubated for 72 hr with ciprofloxacin at 80 μg/ml (------) or 20 μg/ml (·····) or no antibiotic (·····). Cell size of fresh cells (*A*) (——) and autofluorescense (*B* and *C*) (——) of cells not conjugated was also included. The PBLs were stained with CD 25 and OKT-9 antibodies. FSC (forward scattering) expresses the relative cell size. The *y* axes represent the relative number of cells.

The transferrin receptor showed the same intensity of expression on lymphocytes exposed to 20 μg of ciprofloxacin per ml as on those lymphocytes not exposed to antibiotics. However, with ciprofloxacin at 80 μg/ml, there was a very low expression of transferrin receptor. A less pronounced decrease in the expression of the 55-kDa chain of the IL-2 receptor (Tac antigen) was also demonstrated by CD 25 antibodies after 72 hr of incubation of PHA-stimulated human lymphocytes with ciprofloxacin (80 μg/ml) (Fig. 4*C*). On the other hand, with ciprofloxacin at 20 μg/ml, the expression of CD 25 was enhanced as compared to lymphocytes stimulated with PHA but not exposed to ciprofloxacin.

To provide further evidence of an altered expression of the IL-2 receptor by ciprofloxacin, high-affinity receptors for IL-2 were measured by an assay with radiolabeled IL-2. PHA-stimulated lymphocytes incubated for 72 hr with ciprofloxacin at 5 μg/ml demonstrated 1950 receptor sites per lymphocyte, which was about the same as lymphocytes not exposed to ciprofloxacin (Table 3). In contrast, lymphocytes exposed to ciprofloxacin at 20 μg/ml showed 5000 sites per ml and those incubated with 80 μg/ml showed 1200 sites per cell. Thus, our data demonstrate that ciprofloxacin causes two different effects on the IL-2 receptor system. At high concentrations (80 μg/ml), there is a decrease and, at moderate concentrations (20 μg/ml), there is an increase in IL-2 receptors.

DISCUSSION

Our results show that a new group of antibacterial drugs, the 4-quinolones, in concert with a lectin PHA are powerful stimulators of IL-2 production. This was demonstrated both by an increased IL-2 activity in culture supernatants and by increased IL-2 mRNA in PHA-stimulated lymphocytes when exposed to ciprofloxacin, which is one of the most powerful new 4-quinolones. In addition, at moderate concentrations of ciprofloxacin (20 μg/ml), there was an increase in high-affinity receptors for IL-2 as well as the 55-kDa antigen detected by the monoclonal antibody CD 25. However, a very high concentration of ciprofloxacin (80 μg/ml) caused a reduction of the same receptor. At the same concentration of ciprofloxacin, the transferrin receptor was almost completely abolished and cell size did not increase. None of these effects on IL-2 or its receptor was produced by the 4-quinolones in the absence of PHA.

Table 3. High-affinity receptors on PHA-stimulated human lymphocytes incubated with and without ciprofloxacin

Ciprofloxacin, μg/ml	IL-2 receptor sites per cell	Affinity, pM
0	2000	19
5	1950	24
20	5000	31
80	1200	21

Normal human lymphocytes (10^6 cells per ml) were incubated with ciprofloxacin for 72 hr and after washing and additional incubation to facilitate dissociation of endogenously bound IL-2, IL-2 high-affinity receptors were determined with radiolabeled IL-2.

Our results on IL-2 were quite unexpected considering the fact that we previously have reported that ciprofloxacin inhibits cell growth by ≈50% at 20 μg/ml and completely at 80 μg/ml. In addition, immunoglobulin secretion by human lymphocytes stimulated by pokeweed mitogen or Epstein–Barr virus was inhibited 50% with ciprofloxacin at 5 μg/ml and almost completely at 20 μg/ml. In addition, all new 4-quinolones tested at 1.5–25 μg/ml caused an increased uptake of radiolabeled thymidine after 3–5 days of incubation with PHA-stimulated human lymphocytes (3, 4).

The molecular target for 4-quinolones in human lymphocytes causing increased IL-2 production was not revealed by our studies. A selective IL-2 gene expression resulting from a toxic effect by the 4-quinolones seems to be an unlikely explanation for the apparent discrepancy between the increased IL-2 production and the inhibited lymphocyte cell functions. The similarities with the action of the tumor-promoting phorbol ester, PMA or PDB, which stimulates protein kinase C, has to be pointed out. Optimal IL-2 mRNA induction requires the concerted action of PMA or PDB and a lectin like PHA or monoclonal antibodies directed against the T3 antigen complex (20, 21, 27–30). In the present study, ciprofloxacin induced increased IL-2 production in the Jurkat cell line when substimulatory concentrations of PMA and antibodies directed toward the T3 antigen complex were used. However, by analysis of phosphorylation of endogenous protein kinase C substrates, we were unable to detect any stimulatory effect by ciprofloxacin on this kinase.

The lymphokine IL-2 has a short half-life ($t_{1/2}$, 3–5 min) in humans, which is consistent with its role as a transient signal within the immune system, a signal that must be cleared to avoid chronic and possible deleterious stimulation (31). In unstimulated normal human blood lymphocytes, IL-2 mRNA is undetectable but stimulation with mitogens causes its rapid accumulation (32). In activated human PBL, IL-2 mRNA levels decline rapidly on removal of the inducing agents, indicating that transcription continues only as long as the activating signal is present. In addition, it has been suggested that an A+U-rich untranslated region in IL-2 mRNA and in the mRNA of other mediators of inflammation, and in a few other mRNAs, is responsible for an instability (33, 34). IL-2 mRNA degradation presumably by a labile RNase, has been reported to be selectively inhibited by cycloheximide and actinomycin D, inhibitors of protein and RNA synthesis, respectively (33). This leads to superinduction. Quinolones may inhibit individual genes at concentrations lower than

those inhibiting gyrase activity (35, 36). It is possible that IL-2-regulating mechanisms are particularly sensitive to ciprofloxacin in eukaryotic cells.

In general, clinical side effects of the new 4-quinolones are mild to moderately severe and discontinuation of therapy because of drug toxicity is necessitated in only 1–3% of patients (2). Central nervous system side effects are reported by 1–5% of patients. Stimulatory effects may occur because quinolones inhibit receptor binding of γ-aminobutyric acid, an inhibitory transmitter (37). However, this effect was mainly apparent at high concentrations of 4-quinolones. Side effects of high dose IL-2 administration also involve the central nervous system in a rather high number of patients (38, 39). Considering the findings in this report that 4-quinolones, even at the clinically achievable concentration of 5 μg/ml, increase the IL-2 level and at high concentrations dramatically influence IL-2 production in PHA-stimulated human lymphocytes *in vitro*, it is possible that effects of new 4-quinolones on the central nervous system are secondary to their ability to mediate increased IL-2 production in stimulated T cells.

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