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Limited Effects of Temafloxacin Compared with Ciprofloxacin on T-Lymphocyte Function

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Temafloxacin increased interleukin-2 production and mRNA levels and enhanced thymidine incorporation in stimulated lymphocyte cultures. Gamma interferon mRNA levels were unaffected. Temafloxacin also stimulated interleukin-2 gene induction, as revealed in a chloramphenicol acetyltransferase reporter gene system. However, temafloxacin exerted significantly weaker effects in these respects than did ciprofloxacin.

Temafloxacin, a 1-difluorophenyl-6-fluoroquinolone antibiotic, has a wide spectrum of antibacterial activity including effects on *Legionella* spp., *Mycoplasma pneumoniae,* and *Chlamydia trachomatis* (1, 9, 10, 19). Although the pharmacokinetic properties of temafloxacin are superior to those of other fluoroquinolones (5, 16), the drug was recently withdrawn from the market owing to its severe side effects. Approximately 1 of 3,500 patients receiving temafloxacin has been estimated to manifest serious adverse drug reactions (4, 17, 18, 26). Several deaths occurred, and a number of patients required hemodialysis and erythrocyte transfusions.

Ciprofloxacin as well as ofloxacin and other fluoroquinolones are known to interfere with immunological function both in vivo and in vitro (for a review, see reference 27). At concentrations of 1.6 to 50 μg/ml, fluoroquinolones cause a significant increase in the uptake of [^{3}H]thymidine into the DNA of mitogen-stimulated human peripheral blood lymphocytes (PBLs) (3, 6–8, 29). The mechanism responsible for the increased thymidine incorporation by fluoroquinolone-treated PBLs is unknown. Moreover, we and others have recently reported interleukin-2 (IL-2) production to be markedly increased in phytohemagglutinin (PHA)-stimulated PBLs incubated in the presence of fluoroquinolones (21, 23, 29, 32), the increase being due to enhanced IL-2 transcription and the resulting higher levels of IL-2 mRNA (22, 25). Parallel to that of IL-2, increased gamma interferon (IFN-γ) production has been detected in PBL cultures incubated with ciprofloxacin or ofloxacin (11, 24, 32). Interestingly, ciprofloxacin enhances the repopulation of murine hematopoietic organs in sublethally irradiated mice, possibly by promoting IL-3 and granulocyte-macrophage colony-stimulating factor production (14). However, the possible effects of temafloxacin on immune system functions have not been explored.

Preservative-free temafloxacin (Abbott, Chicago, Ill.), ciprofloxacin (Bayer, Wuppertal, Germany), and PHA (Wellcome, Dartford, England) were dissolved in RPMI 1640. PBLs, isolated from buffy coats or from heparinized blood from healthy donors, were incubated at a density of 10^6/ml (21). To quantify DNA synthesis, PBLs were pulsed with [*methyl*-^{3}H]thymidine during the final 18 h of incubation. The biological activity of IL-2 was analyzed as described previously (21). For cell number determination, PBLs were counted in a Bürker chamber; trypan blue was used to exclude dead cells.

Northern (RNA) blots were prepared as described elsewhere (24). Autoradiographs were quantified by scanning laser densitometry. Relative intensities were obtained by dividing all mRNA levels by the corresponding values for triosephosphate isomerase (TPI) mRNA. The IL-2-, IFN-γ-, and TPI-specific cDNA probes and the labeling reaction have been described previously (24). For transfection experiments, a plasmid containing the IL-2 enhancer and promoter region (635 bp) linked to the reporter gene chloramphenicol acetyltransferase (CAT) in conjunction with a simian virus 40 (SV40) enhancer element was used (SV–IL-2–CAT) (15). A plasmid containing the mouse metallothionein promoter and enhancer linked to the SV40 enhancer and the CAT gene (MT-CAT-SV) was used as a control. Transfections and CAT assays were carried out as described elsewhere (see reference 15 and the references therein). After the transfections, the human T-cell lymphoma cell line Jurkat was incubated for 45 h before harvesting. Cells were induced with 1 μg of PHA per ml and 50 ng of phorbol myristate acetate per ml during the last 15 h of incubation, after which extracts were prepared. The CAT reaction was performed in a mixture that included cell extract, ^{14}C-labeled chloramphenicol, and acetyl coenzyme A. After incubation, the products were separated from the substrate by thin-layer chromatography. For quantification, areas containing the acetylated and nonacetylated forms of ^{14}C-labeled chloramphenicol were cut out and measured in a scintillation counter. In all experiments, Student's *t* test for paired data was used for statistical calculations; *P* values of less than 0.05 were considered statistically significant.

To ascertain whether temafloxacin interfered with thymidine uptake by human lymphocytes, PHA-stimulated PBLs were incubated with temafloxacin (2.5 to 80 μg/ml) added at the initiation of culture. As can be seen from Fig. 1A, temafloxacin and ciprofloxacin at 5 μg/ml each increased thymidine uptake 1.4- and 2.3-fold, respectively at 120 h. Temafloxacin (80 μg/ml) increased thymidine uptake twofold at 96 h and fourfold at 120 h. In contrast to temafloxacin, ciprofloxacin at 80 μg/ml almost completely inhibited thymidine incorporation (Fig. 1B). The discrepancy in thymidine uptake between temafloxacin and ciprofloxacin at high concentrations was also manifest when lymphocyte proliferation was investigated at 96 h of incubation; ciprofloxacin abolished cell proliferation, whereas temafloxacin exerted a much weaker inhibitory effect (Fig. 1C and D).

IL-2 production was analyzed in lymphocyte cultures incubated in the presence of temafloxacin. A dose-dependent response was clearly manifest (Fig. 2). At 80 μg/ml and 72 h,

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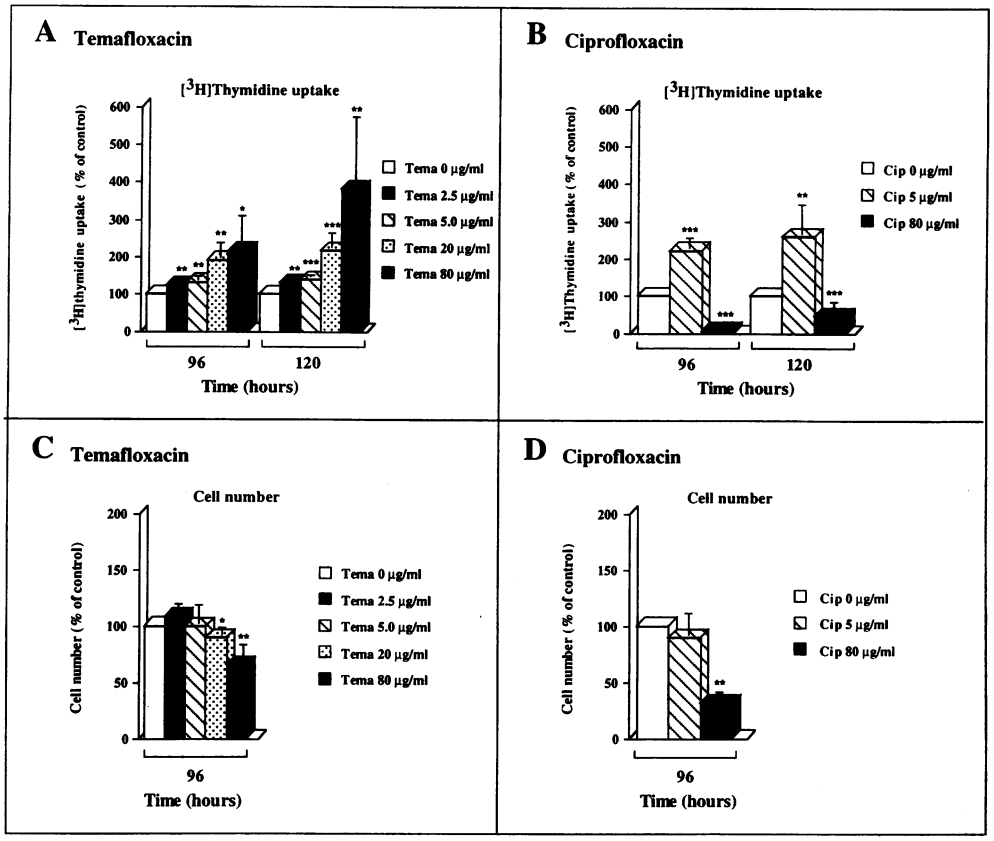

FIG. 1. Effects of temafloxacin (Tema) versus those of ciprofloxacin (Cip) on [^{3}H]thymidine incorporation and cell proliferation in PHA-stimulated human PBLs. (A and B) Temafloxacin or ciprofloxacin was added together with PHA (1 μg/ml) at the initiation of culture. Lymphocytes were pulsed with [^{3}H]thymidine during the last 18 h of incubation after 4 and 5 days. Thymidine incorporation in control cells was 123,430 cpm (96 h) and 70,688 cpm (120 h). (C and D) Cell number was counted after 96 h in lymphocyte cultures incubated with temafloxacin or ciprofloxacin. Each panel shows the means from three experiments with six different blood donors. Error bars indicate standard deviations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

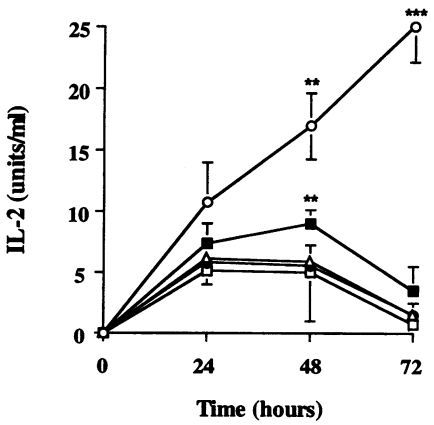

FIG. 2. IL-2 production in lymphocyte cultures incubated with temafloxacin at 80 (○), 20 (■), 5.0 (△), 2.5 (●), and 0 (□) μg/ml. Temafloxacin was supplemented together with PHA at the initiation of lymphocyte culture. Results for samples from six different blood donors are shown. Error bars indicate standard deviations. **, $P < 0.01$; ***, $P < 0.001$.

temafloxacin increased IL-2 production by up to 25 units/ml, whereas 0.1 unit/ml was detected in control cells not exposed to any antibiotic. In contrast, ciprofloxacin (80 μg/ml) enhanced IL-2 synthesis by more than 150 units/ml (data not shown). At 24 h of incubation, temafloxacin and ciprofloxacin (both at 5 μg/ml) enhanced IL-2 production by 20 and 90%, respectively, compared with that in controls. To further investigate the IL-2 increase in the presence of temafloxacin, total RNA was isolated and Northern blots were performed. Blots were hybridized with specific probes for IL-2, IFN-γ, and TPI; TPI was considered not to change its intensity during the cell cycle (24). The observed IL-2 increase (Fig. 2) in temafloxacin-treated PBLs was also detectable at the mRNA level (Fig. 3). Temafloxacin (80 μg/ml) enhanced steady-state IL-2 mRNA levels three to six times in comparison with that in control cells unexposed to antibiotics, but at 20 μg/ml, temafloxacin did not significantly affect IL-2 mRNA levels. In contrast, ciprofloxacin markedly increased IL-2 mRNA concentrations in parallel with the increased IL-2 production. IFN-γ mRNA was also influenced by ciprofloxacin, but temafloxacin manifested only a slight effect on IFN-γ mRNA levels at the highest concentration tested (80 μg/ml) and at 72 h (Fig. 3).

The increased IL-2 mRNA levels suggested an enhanced transcription rate or a decreased IL-2 mRNA degradation in PBLs incubated with the two fluoroquinolones. We investigated the possible involvement of transcriptional regulation in

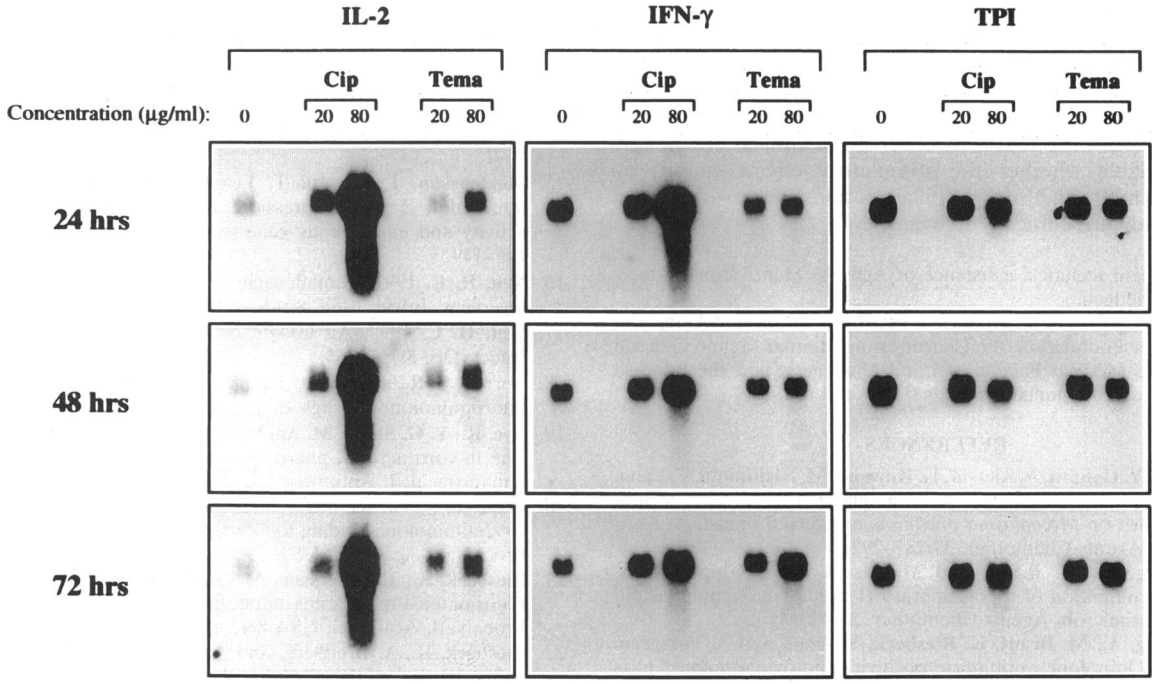

FIG. 3. Effects of temafloxacin and ciprofloxacin on IL-2 and IFN-γ mRNA. PHA-stimulated PBLs were incubated for 24 to 72 h with ciprofloxacin (20 and 80 μg/ml) or temafloxacin (20 and 80 μg/ml); the results were compared with those for control cells unexposed to any fluoroquinolone (lanes 0). Drugs were added at the initiation of culture. The data presented are from a single RNA preparation, but are representative of the results of three independent experiments.

the induction of IL-2 expression in the presence of temafloxacin or ciprofloxacin. Jurkat cells were transiently transfected with a plasmid containing the IL-2 promoter region and CAT gene in conjunction with an SV40 enhancer (SV–IL-2–CAT) (Fig. 4). Temafloxacin at 80 μg/ml increased CAT expression 1.4-fold, i.e., a weaker response than the 3.2-fold increase observed in Jurkat cells incubated with ciprofloxacin (80 μg/ml). To exclude any interference of ciprofloxacin or temafloxacin with the SV40 enhancer element and the CAT gene, experiments were also performed with the plasmid MT-CAT-SV; neither fluoroquinolone was found to affect CAT activity (data not shown), thus confirming the selectivities of temafloxacin and ciprofloxacin for the IL-2 enhancer region.

We showed that ciprofloxacin exerts greater stimulatory effects than temafloxacin on T-lymphocyte functions. At high concentrations, however, ciprofloxacin was considerably more toxic than temafloxacin to lymphocytes, as demonstrated by analysis of cell proliferation and thymidine incorporation. The potency of temafloxacin (IL-2 production and thymidine incorporation) was comparable to that of norfloxacin (3, 21). The antibacterial target of fluoroquinolones is the gyrase-DNA complex (20, 28, 31). In parallel, the eukaryotic counterpart to gyrase, topoisomerase II, is also affected by fluoroquinolones, although at higher drug concentrations (2, 13). Interestingly, the presence of topoisomerase II is also a crucial factor in gene transcription (30). One possible explanation of the effects of fluoroquinolones on cytokine production may be that they induce genotoxic stress secondary to topoisomerase II inhibition, which is followed by transcription factor activation (12). The difference between temafloxacin and ciprofloxacin regarding IL-2 production and thymidine incorporation may thus be explained in part by putative differences in their inhibitory activities upon eukaryotic topoisomerases. Because temafloxa-

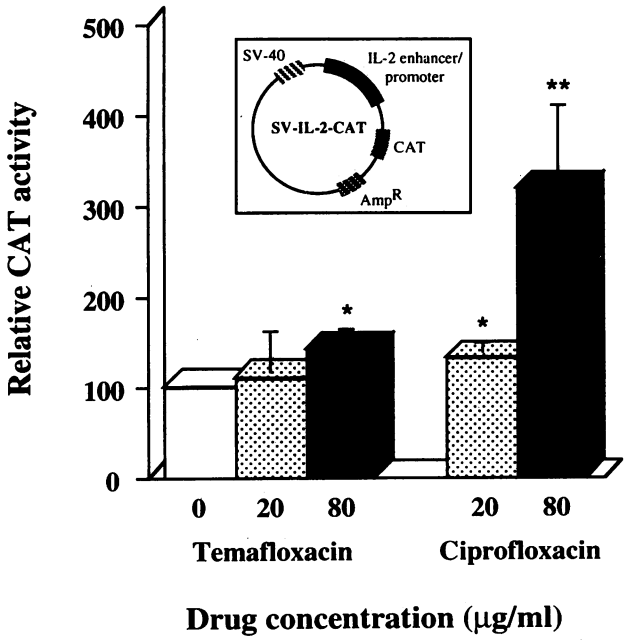

FIG. 4. Dose-dependent IL-2 gene induction in transfected Jurkat cells in the presence of temafloxacin or ciprofloxacin. The SV–IL-2–CAT construct is also shown. Three independent experiments were performed at every concentration. Error bars indicate standard deviations. *, $P < 0.05$; **, $P < 0.01$.

cin inhibited cell proliferation only slightly, perhaps owing to its modest effect on eukaryotic topoisomerase II, the stimulatory effect on IL-2 production was limited. In conclusion, the effects of temafloxacin on T-lymphocyte functions are weaker than those of ciprofloxacin and are most likely not responsible for the adverse drug reactions observed in clinical practice. It is questionable whether the stimulatory effects caused by temafloxacin at high drug concentrations observed in the present study are clinically relevant.

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