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Ciprofloxacin-Induced Inhibition of Topoisomerase II in Human Lymphoblastoid Cells

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The antibacterial activities of the fluorinated 4-quinolones (e.g., ciprofloxacin) have been ascribed to a marked inhibition of bacterial DNA gyrase. In contrast, the influence on purified mammalian DNA enzymes, including topoisomerases, has been reported to be several orders of magnitude weaker, occurring at concentrations higher than 100 μg of ciprofloxacin per ml. In this study, using a nondenaturing filter elution method, a marked induction of double-strand DNA breaks in human lymphoblastoid cells exposed to 80 μg of ciprofloxacin per ml was seen. The proportion of single-strand versus double-strand DNA breaks was similar to that seen with the topoisomerase II inhibitory antitumor agent VP-16. The cellular recovery was more rapid after treatment with ciprofloxacin than after treatment with VP-16, displaying a normal elution profile within 15 min at 37°C (60 min for VP-16). These data indicate that ciprofloxacin has an effect on intracellularly located topoisomerase II in humans.

The antibacterial activities of fluorinated 4-quinolones have been ascribed to the binding of these drugs to DNA (12), leading to a marked inhibition of bacterial DNA gyrase (6). In contrast, the influence on mammalian DNA topoisomerases is several orders of magnitude weaker, as measured by a purified eukaryotic topoisomerase II (topo II) plus plasmid DNA assay (5, 8). In those studies an inhibitory influence on the catalytic function of topo II by ciprofloxacin was noted at minimally effective concentrations of 140 μg/ml (5) or 150 μg/ml (8). Also, topo II-dependent DNA breakage has been assayed by using a plasmid system; cleavage of nonradioactively labeled plasmid DNA was not seen at ciprofloxacin levels up to 1,000 μg/ml, whereas in a more sensitive assay with radioactively labeled plasmid, cleavage was observed at 120 μg/ml (5).

In the present study we investigated the effects of ciprofloxacin on intracellularly located topo II using a human lymphoblastoid cell system. The previously reported (3) cellular DNA breakage associated with ciprofloxacin was determined to be due mainly to the introduction of double-strand DNA breaks (DSBs), indicating a causative role of topo II (13). The proportion of single-strand DNA breaks (SSBs) versus DSBs was similar to that seen with the epipodophyllotoxin antitumor agent VP-16 (13, 14). However, the cellular recovery from the DNA damage was more rapid with ciprofloxacin than with VP-16, displaying a normal elution profile within 15 min at 37°C (60 min for VP-16).

MATERIALS AND METHODS

Drugs. Fresh solutions of preservative-free ciprofloxacin (Bayer, Wuppertal, Federal Republic of Germany) were used. Etoposide (VP-16) was obtained from Bristol-Myers (Syracuse, N.Y.) as a concentrated solution for infusion (20 mg/ml).

UV irradiation. A total of 10^6 cells suspended in 4 ml of phosphate-buffered saline (pH 7.4) in a 5-cm-diameter plastic dish were exposed (dish lid off) to radiation from an ordinary laboratory hood UV tube (GTE Sylvania G30 T8) emitting 1.0 W of UVC per m^2 at a distance of about 30 cm (as measured with a UV-X Radiometer, Ultraviolet Products Inc., San Gabriel, Calif.).

X-ray irradiation. X rays were delivered at a dose rate of 8 Gy/min with a 6-MeV Clinac 1800 linear accelerator (Varian), with the cells kept on ice in a few milliliters of phosphate-buffered saline in a plastic dish.

Cells. The human lymphoblastoid cell line Raji was cultivated at 37°C in a humidified 5% CO_2 atmosphere in RPMI 1640 medium (GIBCO Co., Paisley, Scotland) supplemented with 10% fetal bovine serum, L-glutamine, and gentamicin (12 μg/ml). For cell number determination, Raji cells were counted manually by using Bürker chambers. The cell number doubling time was 20 to 24 h up to a concentration of about 1.2×10^6 cells per ml. As determined by a standard immunofluorescence assay, the cells were free of mycoplasmas.

Elution of DNA. Raji cells were labeled with [^{3}H]thymidine (1 μCi; 0.125 μCi/ml of growth medium; specific activity, 78 Ci/mmol; NET-027Z; Du Pont, New England Nuclear, Boston, Mass.). Labeling was carried out from a starting cell concentration of 2×10^5/ml over a period of 24 h. The isotope was then removed by washing, the cells were resuspended in complete medium, and the appropriate drug was added so that it was present during another 2 h of growth. The cells (about 10^6) were then pipetted onto a 25-mm polycarbonate filter (pore size, 2 μm [Nuclepore Corp., Pleasanton, Calif.] or 3 μm [Millipore Corp., Bedford, Mass.]) and washed once with phosphate-buffered saline (always chilled on ice). Immediately thereafter, the cells were lysed with a 2% sodium dodecyl sulfate–20 mM EDTA (pH 9.5) solution at room temperature and washed with 20 mM EDTA (pH 9.5). Finally, tetraethylammonium hydroxide (pH 12.0 or 9.2) was slowly pumped through the filter and 5-ml fractions were collected, mixed with 2 volumes of OptiPhase (LKB, Bromma, Sweden), and measured by liquid scintillation counting. The activity remaining on the filter was recovered as described by Kohn (9). The percentage of DNA retained on the filter was calculated by dividing the counts per minute on the filter by the total counts per minute (defined as counts per minute on the filter plus all of the eluted tetraethylammonium hydroxide; only a small

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TABLE 1. DNA double-strand breaks in human lymphoblastoid cells[a]

Cell treatment	No. of expts	% DNA retained on filter[b]
None	18	94 ± 1
Ciprofloxacin		
5 μg/ml	7	94 ± 3
20 μg/ml	7	92 ± 3
80 μg/ml	11	55 ± 4
VP-16		
1.25 μg/ml	4	61 ± 8
5 μg/ml	4	53 ± 4
20 μg/ml	4	41 ± 11
80 μg/ml	4	35 ± 10
X rays		
3,000 rads	2	61 ± 5
10,000 rads	2	39 ± 16

[a] Breaks were measured by filter elution at pH 9.2.
[b] The standard error of the mean is given.

amount of radioactivity was usually recovered in the washing and lysis solutions).

RESULTS

Human lymphoblastoid Raji cells were cultivated for 24 h in [^{3}H]thymidine-containing medium, after which they were washed and chased for 2 h in ^{3}H-free medium to which ciprofloxacin was added. The cells were then deposited and lysed on a filter. Thereafter, the DNA was eluted at either one of two pHs: pH 12.0, giving denaturing conditions that separate the two DNA strands from each other, or pH 9.2, providing nondenaturing conditions. The smaller the DNA fragment, the more likely it is to be passed through the filter pores (see references 2 and 9 for theoretical considerations). Thus, elution at pH 12.0 reflects the number of SSBs and DSBs present in the DNA, whereas at pH 9.2 only DSBs cause an increased elution rate.

The results of elution under nondenaturing conditions are shown in Table 1. With untreated cells, about 95% of the cellular DNA was retained on the filter at the end of the elution. Ciprofloxacin at 80 μg/ml was found to cause about 50% of the DNA to become eluted from the filter, indicating induction of DSBs, whereas a very weak effect (statistically nonsignificant) was seen at 20 μg/ml. The potent antitumor agent VP-16 caused a large amount of DNA breakage at 1.25 μg/ml, increasing moderately up to 80 μg/ml, at which two-thirds of the cellular DNA was eluted. X rays, which have been used by others (2) as a prototype agent in DSB studies, were observed to strongly increase the DNA elution at a dose level higher than 1,000 rads (with 3,000 rads giving 39% elution and 10,000 rads giving 61% elution).

The results of representative experiments are plotted in Fig. 1, with the respective pHs given side by side. Ciprofloxacin at 40 μg/ml with elution at pH 12.0 gave a DNA elution rate similar to that for ciprofloxacin at 80 μg/ml with elution at pH 9.2 (Fig. 1A and B). This shows that a large proportion of the breaks observed at pH 12.0 were double-stranded. VP-16 was found to give a ratio of SSB:DSB similar to that of ciprofloxacin, since 1.25 μg/ml at pH 12.0 gave an elution profile resembling that of 5 μg/ml at pH 9.2 (Fig. 1C and D). A similar proportion of SSBs versus DSBs (see reference 9) was found by others for ellipticine (10) and

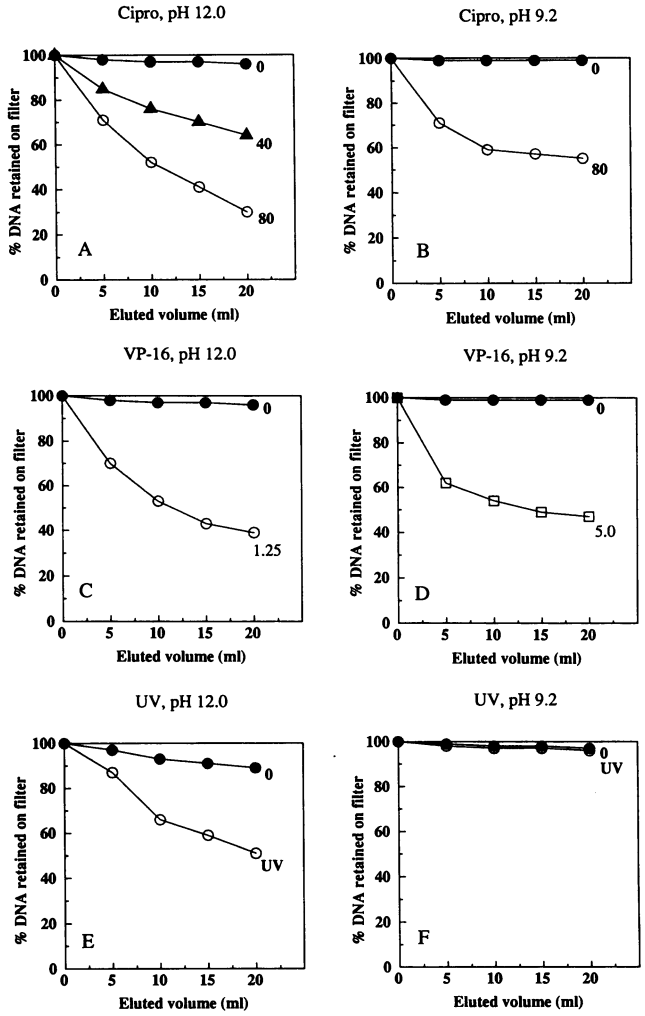

FIG. 1. Filter elution of DNA from drug-treated human lymphoblastoid Raji cells. After the cellular DNA was labeled with [^{3}H]thymidine, the cells were treated with the indicated drug (ciprofloxacin [Cipro] or VP-16) for 2 h, lysed on the filter, and finally, assayed for the presence of SSBs (pH 12.0) or DSBs (pH 9.2). After UV treatment, the cells were kept at 37°C for 30 min to allow DNA repair breaks to be formed.

VP-16 (14), using the same elution technique as that used in the present study.

In order to document the specificity of the pH 9.2 elution method in terms of detecting DSBs but not SSBs, the experiments for which the results are shown in Fig. 1E and F were done. The cells were exposed to short-wave UV radiation and then incubated at 37°C for DNA excision repair to take place, during which process SSBs are known to be enzymatically induced (4). At pH 12.0 (Fig. 1E) a clear breaking effect was seen, whereas little influence was seen at pH 9.2 (Fig. 1F).

After treatment with ciprofloxacin or VP-16, incubation of the cells in drug-free medium was seen to relieve them from the influence of DSBs (Fig. 2). Recovery from ciprofloxacin was already complete at the first observation time (15 min). By using VP-16 at a concentration that gave a level of DNA breakage similar to that given by ciprofloxacin, recovery was only partial 15 min after drug removal. However, it was virtually complete at 1 h postincubation.

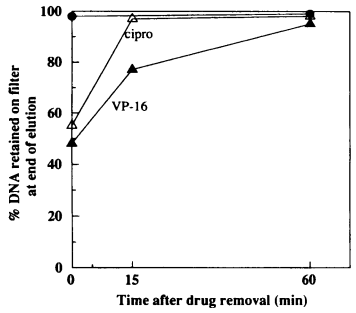

FIG. 2. Recovery of Raji cells from DNA DSBs. After drug treatment (80 μg of ciprofloxacin [cipro] per ml and 1.25 μg of VP-16 per ml), the cells were incubated in drug-free medium for the indicated times before being lysed on the elution filter. Filled circles denote cells to which no drug was added.

DISCUSSION

Inhibition of topo II is usually considered to be of major importance for (i) the antibacterial effect of fluorinated 4-quinolones, including ciprofloxacin (5), and (ii) the antitumor effect of a group of cytotoxic compounds, including adriamycin, *m*-AMSA [4′-(9-acridinylamino)methane-sulfon-*m*-anisidide], ellipticine, and VP-16 (10, 13, 14). With regard to this similarity between these two drug classes and to the wide clinical usage of 4-quinolone antibiotics, it is of importance to assess any possible genotoxic or oncogenic influences of the 4-quinolones. Commonly, the 4-quinolones are considered to render to the patients only a negligible toxic influence (11). However, the literature on this subject has presented controversial results (e.g., see references 3, 6, 7, and 11), with some in vitro tests giving an indication of genotoxic damage formation and others providing no such evidence. We have previously reported that human lymphoblastoid cell DNA is broken by ciprofloxacin in culture (3), illustrating that this drug has the capacity to affect cellular DNA in vitro. The results of the present study indicate that the major part of ciprofloxacin-induced DNA breakage can also be seen under nondenaturing conditions. This reflects the induction of DSBs (2) and suggests that topo II is involved in the generation of these breaks (13).

The relevance of this DNA break induction, in terms of side effects in treated patients, is dependent on the mechanism behind the DNA breakage. Since the cells are chased in ^{3}H-free medium before they are assayed, we probably did not measure the breaks that occurred transiently during the normal replication process. Other possible mechanisms include formation of breaks during enzymatic excision repair of DNA damage (e.g., UV dimers and chemical adducts). Also, the direct induction of breaks, i.e., without enzyme mediation, is well documented (e.g., X rays, long-wave UV, and methanesulfonates).

Therefore, which mechanism of DNA breakage is the most likely to become activated by ciprofloxacin? Even after documentation of topo II inhibition, we are still left with at least two alternatives. Is topo II inhibited (or, rather, "frozen" in a stage between DNA break formation and resealing) while executing some of its normal activities during replication and transcription or while taking part in enzymatic (perhaps recombinogenic; see reference 1) processing of a ciprofloxacin-DNA complex (DNA binding has been shown by Shen et al. [12])? Whichever of these two alternative mechanisms applies, it is difficult to judge the influence on DNA as being of insignificant relevance; topo II is an enzyme that acts during such basic processes as replication and transcription of DNA; it has been reported to mediate illegitimate recombination of possible oncogenic importance (1); topo II inhibition has been considered to be the mechanism of a toxic and mutagenic class of antitumor compounds (10, 13, 14). In this context, the observation by Gootz et al. (5), who used a novel quinolone, CP-67015, of a correlation between a positive outcome of various genotoxic tests (chromosome breakage and hypoxanthine-guanine phosphoribosyltransferase gene mutations) and a high level of topo II-mediated plasmid DNA breakage should be noted. However, it must be stressed here that CP-67015 stands out from other quinolones in that it is a markedly more potent inducer of topo II-mediated DNA cleavage.

The findings of the present study of an inhibitory influence on human intracellular topo II at a low dose level of between 20 and 80 μg of ciprofloxacin per ml may serve to stimulate further research into the action of 4-quinolones on human cells.

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