The Cytotoxic Effects of Fleroxacin and Ciprofloxacin on Transitional Cell Carcinoma In Vitro

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BACKGROUND. There have been few reports concerning the cytotoxic effects of fluoroquinolone antibiotics on transitional cell carcinoma. This investigation was designed to study the cytotoxic effects of fleroxacin and ciprofloxacin on transitional cell carcinoma quantitatively in vitro.

METHODS. Two transitional cell carcinoma cell lines, MBT-2 and T24, were used in this study. The effects of fleroxacin and ciprofloxacin on cell proliferation were determined by counting the number of living cells and by colorimetric MTT assay.

RESULTS. Two fluoroquinolones, fleroxacin and ciprofloxacin, significantly inhibited cell proliferation in a dose-dependent manner at a concentration of 50-800 μg/mL in both cell lines. Compared with the cytotoxic effects of the two antibiotics, the inhibitory activity of ciprofloxacin on cell proliferation significantly exceeded that of fleroxacin in the MBT-2 cell line. However, the two fluoroquinolones did not have significantly different effects on the T24 cell line.

CONCLUSIONS. Fleroxacin and ciprofloxacin significantly affect cell proliferation in transitional cell carcinoma cell lines. The results encourage further study of the possibility of clinical application of some fluoroquinolones to prevent recurrence of urinary bladder tumors because the urinary excretions after oral administration of these drugs are quite high. ***Cancer* 1997; 80:2263–7.**

KEYWORDS: cytotoxicity, fluoroquinolone, fleroxacin, ciprofloxacin, transitional cell carcinoma, MBT-2, T24.

Transitional cell carcinoma of the bladder is superficial at presentation in 70-80% of patients. Patient survival is approximately 90% after 5 years and 80% after 10 years. Due to the excellent prognosis, the initial treatment usually is transurethral resection (TUR) of the tumor, followed by periodic observation alone or in combination with intravesical immunotherapy (bacille Calmette-Guérin) or chemotherapy to prevent recurrence. However, the recurrence rate is very high, and some patients remain nonresponsive despite receiving postoperative adjuvant therapy. Furthermore, tumor progression subsequently is observed in 24- 56% of patients with superficial tumors.[1] More effective adjuvant therapies thus are needed to prevent tumor recurrence after TUR in place of conventional intravesical chemotherapy or immunotherapy.

It is well known that fluoroquinolone antibiotics inhibit bacterial DNA gyrase (DNA topoisomerase type II), and that urinary concentrations of these compounds after oral administration are extremely high. Ciprofloxacin and ofloxacin recently have been shown to have cytotoxic effects on both murine and human transitional cell carcinoma cells in vitro.[2,3] Further studies are warranted because some fluoroquinolone antibiotics may be effective in preventing the recur-

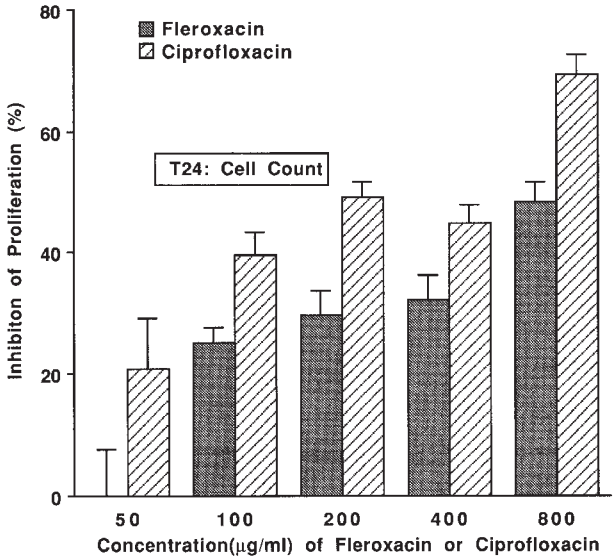

FIGURE 1. The inhibitory effects of fleroxacin and ciprofloxacin on the proliferation of T24 cells as shown by cell counting. Each value is represented as the mean ± standard error of the mean of six cultures.

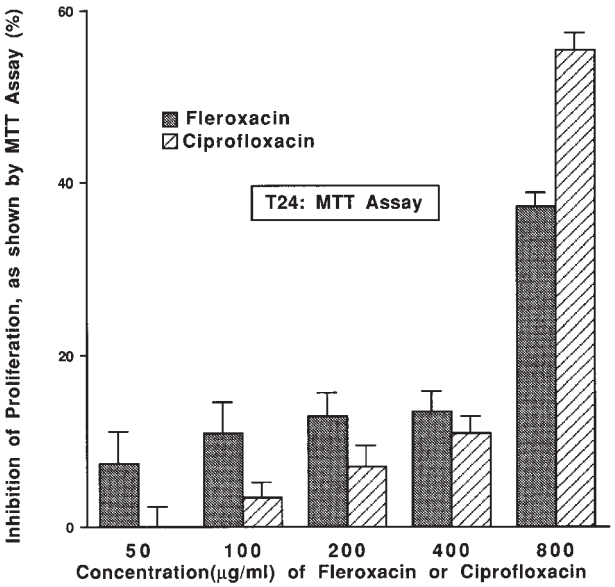

FIGURE 2. The inhibitory effects of fleroxacin and ciprofloxacin on the proliferation of T24 cells determined by the MTT assay. Each value is represented as the mean ± standard error of the mean of six cultures.

rence of bladder tumors. This study was designed to study the cytotoxic effects of a different fluoroquinolone, fleroxacin, in comparison with those of ciprofloxacin on transitional cell carcinoma quantitatively in vitro.

MATERIALS AND METHODS

Culture of Cell Lines

The human transitional cell carcinoma cell line, T24, was obtained from the Japanese Cancer Research Resources Bank. The MBT-2 cell line was derived from a tumor induced by N-[4(5-nitro-2-furyl)-2-thizolyl] formamide in mice by Soloway,[4] and was kindly supplied by the Department of Urology at Tokyo University. Both cell lines were subcultured in minimum essential medium (MEM) (Gibco, Grand Island, NY) containing 10% fetal calf serum (FCS) (Nipro, Osaka, Japan) and 1% antibiotic (penicillin and streptomycin)/antimycotic (amphotericin B) solution (Gibco) at 37 °C in a 5% CO_2 and 95% air atmosphere.

Fluoroquinolone Antibiotics

Fleroxacin (6,8-difluoro-1-[2-fluoroethyl]-1,4dihydro-7-[4-methyl-1-piperazinyl]-4-oxo-quinoline-3 carboxylic acid) was obtained from the Kyorin Pharmaceutical Company (Tokyo, Japan). Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid hydrochloride hydrate), was obtained from Bayer, Yakuhin Ltd. (Osaka, Japan).

Cell Proliferation Assays

The effects of fleroxacin and ciprofloxacin on cell proliferation were determined by counting the numbers of living cells and by the colorimetric MTT assay based on the activities of mitochondrial enzymes in viable cells. To test the effects of fleroxacin and ciprofloxacin by cell counting, both cell lines were passaged into 6-well culture plates at a seeding density of 2.5×10^5 cells/well. After 24 hours, complete medium was removed and changed to MEM containing 10% FCS and fleroxacin or ciprofloxacin at final concentrations of 50, 100, 200, 400, and 800 μg/mL. The living cells then were counted after 24-hour incubation.

Flat 96-well culture plates seeded at a density of 3.3×10^3 cells/well were used to test the effects by MTT assay. These treatments and concentrations of both antibiotics were coordinated with the cell counting method. The colorimetric MTT assay used was similar to that originally described by Mosmann.[6] MTT (Wako) was dissolved at 5 mg/mL in phosphate-buffered saline solution and used. Twenty-four hours after replacement of the medium with fresh MEM containing these fluoroquinolone antibiotics, 10 μL of MTT solution was added directly to the medium and cells were incubated for an additional 2 hours. After removal of the medium, 100 mL of 0.04 M hydrochloric acid in isopropanol was added to each well for solubilization of the formazan crystals, and the optical density of the plates was measured on a microculture plate reader (Corona Electric, MTP-32, Ibaragi, Japan) using

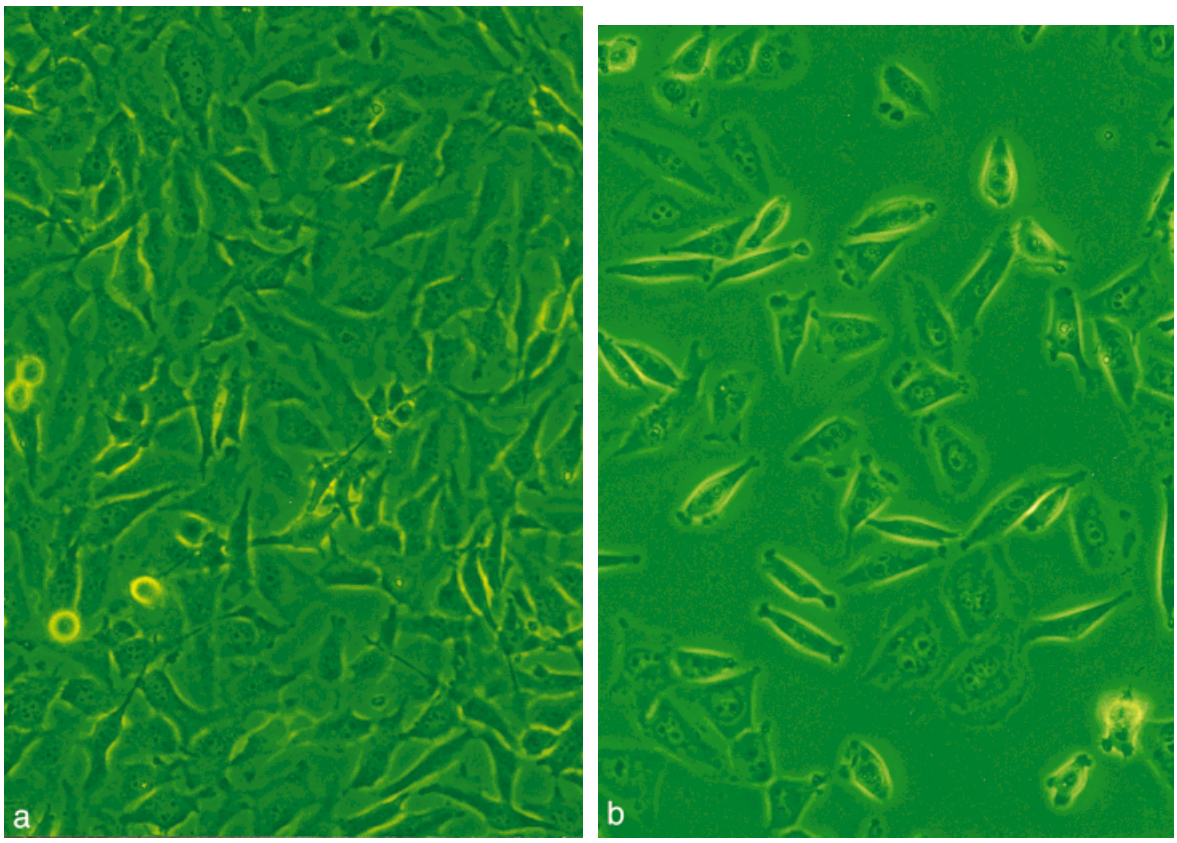

FIGURE 3. (a) MBT-2 in culture with complete minimum essential medium without fluoroquinolone antibiotics (original magnification ×200). (b) MBT-2 in culture after 24-hour growth in the presence of fleroxacin at a concentration of 400 μg/mL (original magnification ×200). The inhibitory activity of fleroxacin on the proliferation of MBT-2 cells is obvious.

a test wavelength of 550 nanometers (nm) and a reference wavelength of 630 nm.

Statistics

Data were compared using the Student's *t* test, and a P value < 0.05 compared with the nontreatment group was considered significant. Values are expressed as the mean ± the standard error of the mean.

RESULTS

T24 Cell Line

The effects of various concentrations of fleroxacin and ciprofloxacin on the proliferation of T-24 cells for 24 hours are shown in Figures 1 and 2, respectively. As determined by the living cell count, fleroxacin at concentrations of 50 μg/mL did not affect cell proliferation significantly (Fig. 1). However, fleroxacin at concentrations of 100-800 μg/mL significantly inhibited proliferation, and the effects were proportional to the concentration of fleroxacin applied (Fig. 1). Ciprofloxacin at concentrations of 50-800 μg/mL significantly affected cell proliferation as shown by cell counting. As determined by the MTT assay, fleroxacin at concentrations of 50-800 μg/mL and ciprofloxacin at concentrations of 100-800 μg/mL inhibited the proliferation of the T24 cell line (Fig. 2).

MBT-2 Cell Line

Figure 3a shows MBT-2 cultured in complete MEM by phase-contrast histology, whereas Figure 3b shows MBT-2 cells exposed for 24 hours to fleroxacin at a concentration of 400 μg/mL. The inhibitory activity of fleroxacin on the proliferation of MBT-2 cells was obvious histologically. As determined by the counting of living cells, fleroxacin at concentrations of 100-800 μg/mL, and ciprofloxacin at concentrations of 50-800 μg/mL significantly inhibited proliferation of the MBT-2 cell line (Fig. 4). The MTT assay indicated that fleroxacin at concentrations of 50 and 100 μg/mL did not affect cell proliferation significantly, whereas at 200-800 μg/mL this agent had significant effects (Fig. 5). The inhibition of cell proliferation with ciprofloxacin measured by the MTT assay was dose-dependent at 50-800 μg/mL (Fig. 5).

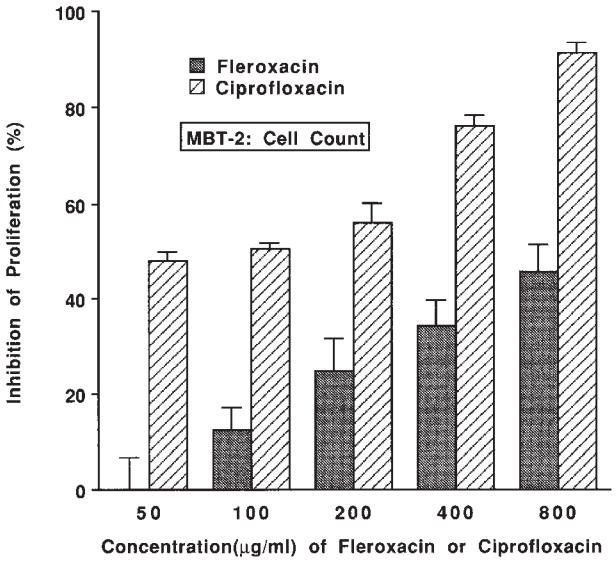

FIGURE 4. The inhibitory effects of fleroxacin and ciprofloxacin on the proliferation of MBT-2 cells, as shown by cell counting. Each value is represented as the mean ± standard error of the mean of six cultures.

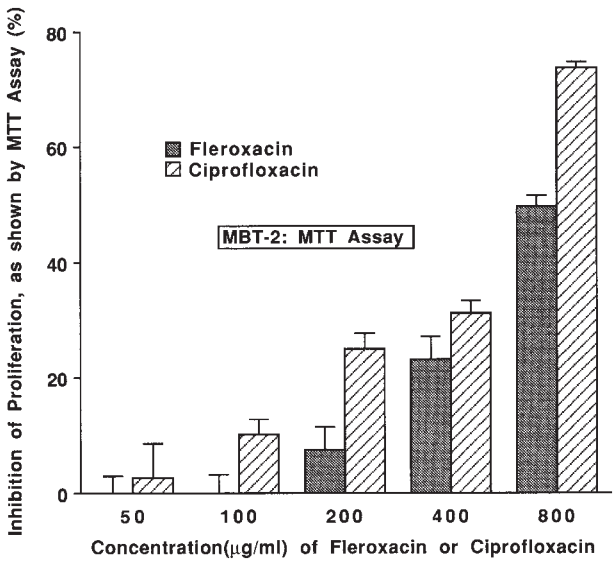

FIGURE 5. The inhibitory effects of fleroxacin and ciprofloxacin on the proliferation of T24 cells as shown by cell counting. Each value is represented as the mean ± standard error of the mean of six cultures.

Comparing the cytotoxic effects of both antibiotics, the inhibitory activity of ciprofloxacin on MBT-2 cells significantly exceeded that of fleroxacin, as shown by the cell number counting and the MTT assay. No distinct differences in inhibitory activity on the proliferation of the T24 cell line were detected between the two fluoroquinolones, because there were some discrepancies in the results of cell counting and the MTT assay.

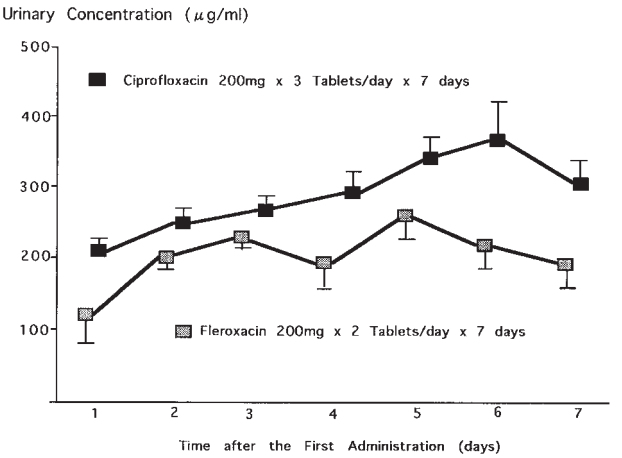

FIGURE 6. Urinary concentrations of fleroxacin and ciprofloxacin after multiple and consecutive oral administration. These data are taken from Nakashima et al.[10] and Kobayashi et al.[11]

DISCUSSION

Fluoroquinolones show bactericidal activity by inhibiting DNA gyrase (topoisomerase type II). Topoisomerase is essential for DNA packaging, transcription, and replication. Hussy et al reported four fluoroquinolones that affected mammalian DNA topoisomerase I, topoisomerase II, and DNA polymerase.[6] They also found that ciprofloxacin was the most potent inhibitor of these enzymes and ofloxacin was the weakest. The research groups of Chu et al.[7] and Yamashita et al.[8] independently demonstrated that antitumor quinolones have a closely related structure: two halogens at C-6 and C-8 and cyclopropyl at N-1 of the quinolone skeleton. Several fluoroquinolones are capable of being used as antitumor agents clinically in the future, and Clement et al.[9] have developed a new antitumor quinolone that showed distinctive activity against murine and human tumors experimentally.

The cytotoxic activities of newer fluoroquinolones on urothelial tumors or transitional cell carcinomas have not been evaluated in detail. Zehavi-Willner and Shalit[2] reported that ciprofloxacin at a concentration of 100 mg/L significantly inhibited the proliferation of human bladder carcinoma cells (BS-5867), and that this effect was reversible at lower doses. However, at higher concentrations (>250 mg/L) of the antibiotic, which exerted a lytic effect on the cells, the antiproliferative effect was not reversible. Seay et al.[3] recently demonstrated similar effects with ciprofloxacin and ofloxacin in three human transitional carcinoma cell lines (TCCSUP, T24, and J82). They also showed that there was no significant differences in cytotoxicity between these 2 agents unless the initial pH of the

medium was adjusted to 5.5. However, the increased cytotoxicity observed at pH 5.5 with ciprofloxacin was at the lowest level (25 μg/mL) compared with that observed when the pH of the medium initially was >6.9. Thus they suggested that the cytotoxic activities might be influenced by the pH of the medium and the effects may be reversible. Unfortunately, we did not examine the effects of pH on the cytotoxicities of fleroxacin and ciprofloxacin against the T24 and MBT-2 cell lines in this study. Fleroxacin was extremely stable from pH 7.5 to 7.6 in room air, even at the highest concentration applied (800 μg/mL). However, fleroxacin was not resolved fully and could be observed as small precipitates in the medium at higher concentrations (>200 μg/mL). The pH of the medium containing ciprofloxacin varied in proportion to the concentration. The pH of the medium containing 50 μg/mL ciprofloxacin was 7.50, and the pH was 7.46, 7.42, 7.34, and 7.20, respectively, at 100 μg/mL, 200 μg/mL, 400 μg/mL, and 800 μg/mL in room air. The media with ciprofloxacin were incubated in a CO_2 incubator for 30 minutes, and the pH increased to >7.5, except at a concentration of 800 μg/mL, at which the pH reached 7.5 after 6 hours of incubation. Therefore, the cytotoxic effects of ciprofloxacin at 800 μg/mL might be enhanced by varying the pH of the culture medium.

In this study, the inhibitory activities measured by cell counting and MTT assay were generally proportional to the concentrations of the two antibiotics. However, there were some discrepancies in the results between the two assays. It was assumed that the reliability of the MTT assay might vary according to the cell number at the time of measurement. In the original MTT method, linearity was obtained from 2×10^2 to 5×10^4 cells/well.[5] In this study, subcultured cells were seeded at a density of 3.3×10^3 cells/well in 96-well cell culture plates, and the cells grew through approximately 5 (T24 cell line) or 10 population doublings (MBT-2 cell line) during 48-hour incubation without any treatment. Thus, the cell numbers probably were sufficient for estimation of cell viability. However, the tendency of lower concentrations of the two fluoroquinolones to show lesser effects were marked when determined by the colorimetric MTT assay. We cannot discuss the reasons for this because the two assays were not performed and analyzed at the same time.

The renal excretion of fluoroquinolones fortunately is quite high. The urinary concentrations after consecutive oral administration of fleroxacin and ciprofloxacin are shown in Figure 6.[10,11] After 7 days of continuous oral administration of 200 mg of fleroxacin twice daily, the urinary concentration exceeded 200 μg/mL from the second day. The levels of renal excretion were >200 μg/mL on the first day after administration of 200 mg of ciprofloxacin 3 times daily. Cytotoxic effects at these concentrations for both agents were observed in the MBT-2 and T24 cell lines. Both fleroxacin and ciprofloxacin already are in wide clinical use as antibiotics. We currently are engaged in large scale experiments to study the inhibition of chemically induced mouse bladder tumors by fleroxacin, and expect to publish the results in the near future. Although further in vitro and in vivo investigations are needed to elucidate the mechanisms by which these fluoroquinolones act as antitumor agents, basic studies such as the current one may provide new insight into prophylactic treatment regimens.

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