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Photostability and Biological Activity of Fluoroquinolones Substituted at the 8 Position after UV Irradiation

MASAHIKO MATSUMOTO,[1]* KANA KOJIMA,[1] HIROYUKI NAGANO,[1] SHUZO MATSUBARA,[1] AND TAKESHI YOKOTA[2]

Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba, Shizuoka 412,[1] and Department of Bacteriology, School of Medicine, Juntendo University, Bunkyo-ku, Tokyo 113,[2] Japan

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Q-35 [1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylaminopiperidine-1-yl)-4-oxoquinoline-3-carboxylic acid], a fluoroquinolone, has absorbance peaks at 333 and 286 nm. No spectral change was observed even when this aqueous solution was irradiated with 3 J of long-wavelength UV light (UVA) per cm^2. On the other hand, its derivatives, which are unsubstituted (8-H analog) or which are substituted with fluorine at the 8 position (8-F analog), were found to have decreased antibacterial activities with a simultaneous increase in their cytotoxicities when they were degraded in a dose-dependent manner with respect to UVA irradiation. Similar results were observed with the other available fluoroquinolones. Enoxacin and lomefloxacin exposed to 0.3 J of irradiation per cm^2 and norfloxacin, ofloxacin, and ciprofloxacin exposed to 1 J of irradiation per cm^2 underwent absorption spectrum changes, an accompanying decrease in antibacterial activity, and an increase in cytotoxic activity. These results suggest that the introduction of a methoxy group into the 8 position of quinolones plays an important role in the stability of fluoroquinolones against irradiation by UV light.

Fluoroquinolones are antimicrobial agents that are administered orally. Their absorption is quite good, and they have a broad antibacterial spectrum. Consequently, they are clinically used as the antibiotics of first choice for general bacterial infectious diseases, and their efficacies are highly appreciated. However, antibiotics of this class exhibit, although at low frequency, side effects against the digestive and central nervous systems and are known to induce severe photosensitivity on rare occasions (6, 12). In particular, nalidixic acid (NA), a prototype fluoroquinolone, is reported to cause photosensitivity in sporadic cases (1, 3, 4). However, the phototoxic mechanism as the cause of abnormal photosensitivity and its relationship to chemical structures remain unclarified.

Q-35 is a new compound in which the 7 position of the fluoroquinolone ring is substituted by a methylaminopiperidine group and its 8 position is modified with a methoxy group. Its antibacterial spectrum is broad, ranging from gram-positive bacteria to gram-negative bacteria, and it exhibits excellent antibacterial activity against gram-positive bacteria such as multiple-drug-resistant staphylococci and pneumococci (15, 17). Here, we report that when the stability of Q-35 (8-OCH_3) in aqueous solution against UV irradiation was investigated and compared with those of its 8-H and 8-F analogs, compounds with different groups substituted at the 8 position, only Q-35 (8-OCH_3) was found to be resistant to irradiation.

MATERIALS AND METHODS

Compounds. Q-35 (8-OCH_3) [1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylaminopiperidine-1-yl)-4-oxoquinoline-3-carboxylic acid], an analog of Q-35 unsubstituted at the 8 position (8-H), and an analog of Q-35 substituted with fluorine at the 8 position (8-F) were synthesized (19) at the Exploratory Research Laboratories of Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan. Enoxacin (ENX; Dai-Nippon Pharmaceutical Co., Osaka, Japan), norfloxacin (NFLX; Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan), ofloxacin (OFLX; Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), ciprofloxacin (CPFX; Bayer Pharmaceutics, Osaka, Japan), and lomefloxacin (LFLX; Hokuriku Pharmaceutical Co., Ltd., Fukui, Japan) were extracted and purified from marketed tablets.

UVA irradiation. The agents were dissolved in a small volume of 0.025 N sodium hydroxide solution and were then added to 20 mM phosphate buffer solution (pH 7.3) to make a final concentration of 20 μg/ml. Forty milliliters of this solution was transferred to a 50-ml beaker (4.5-cm diameter), and the solution was irradiated from a 15-cm distance with UV light (UVA) while it was stirred with a magnetic bar. The source of UV light was black light fluorescent bulbs (20 W, two bulbs; Toshiba, Tokyo, Japan) which have UV light in a region of UVA (320 to 400 nm). This lamp had a spectroscopic distribution of 300 to 400 nm, with a peak at 352 nm, and its mean irradiation intensity was 2.34 J/cm^2/h (as measured with a UVX-36 sensor; Ultraviolet Products Inc., San Gabriel, Calif.). Preexposure solutions of antibiotics were used as controls, and samples taken at successive intervals were stored in the dark until the measurements were taken.

Analytical methods. Absorption spectra were measured with an automatic spectrophotometer (U-3200; Hitachi, Tokyo, Japan). Quantitation of antibiotics was performed with a high-performance liquid chromatograph (655; Hitachi) with an A-302 ODS column (YMC, Kyoto, Japan), in which the mobile phase contained 5 mM tetrabutylammonium hydrogen sulfate solution (Wako Pure Chemical, Osaka, Japan) in 18, 15, and 13% acetonitrile. The flow rate was 0.8 ml/min, and monitoring was done by determining the A_{286}, A_{282}, and A_{273}.

Assay of antibacterial activity. Aqueous solutions of the fluoroquinolones were tested for their antibacterial activities by the paper disk assay method with *Bacillus subtilis* ATCC

* Corresponding author.

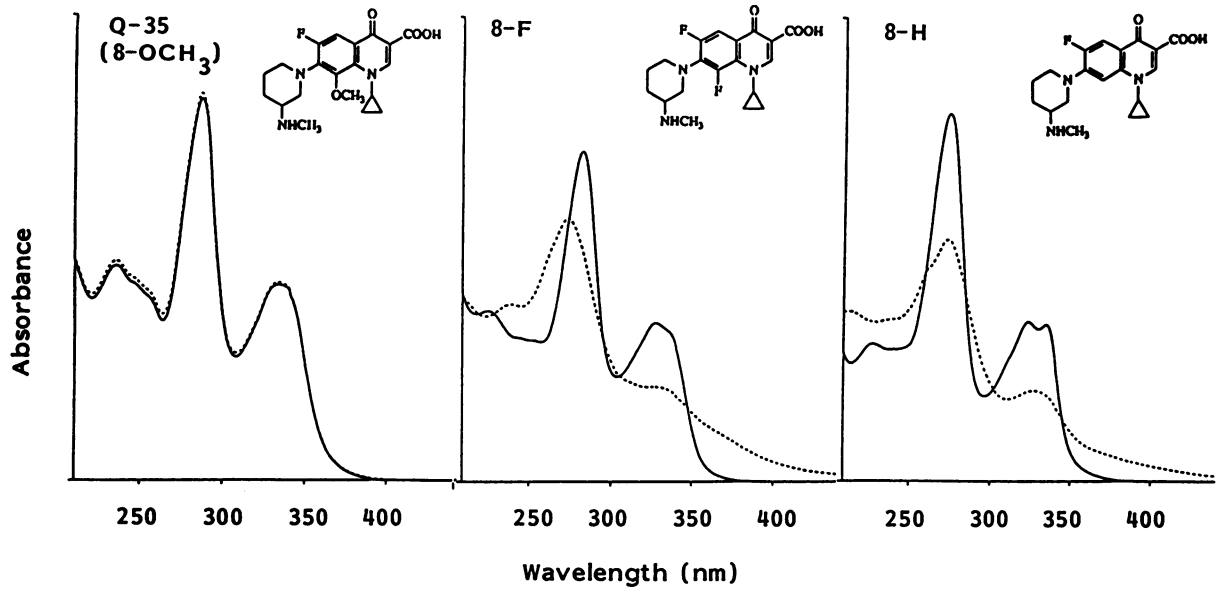

FIG. 1. Change in UV absorption spectra in the solution of 8-substituted fluoroquinolones. Forty milliliters of the solution containing 20 μg of each fluoroquinolone per ml was transferred to a 50-ml beaker, which was stirred with a magnetic bar, and the solutions were irradiated with 3 J of UVA per cm^2. The absorbances of preexposure (——) and postexposure (– – –) solutions were determined with a UV spectrophotometer.

6633 as the test bacterium. Briefly, the bacteria (10^6 CFU/ml) were mixed with 8 ml of a nutrient agar (Difco Laboratories, Detroit, Mich.) and poured into petri dishes. Then, three paper disks (diameter, 8 mm; thickness, 1.5 mm; Advantec, Tokyo, Japan) impregnated with 60 μl of sample antibiotics were placed onto a plate. After overnight culture, the antibacterial activity of the sample was measured and was compared with the standard curve created in the same experiment. The results were expressed as a percentage of the preexposure activity (20 μg/ml).

Assay of cytotoxicity. Inhibition of the proliferation of mouse lymphocytic leukemia cells (L-1210) was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (18). L-1210 cells (1×10^5/ml) were suspended in RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing Hanks' balanced salt solution, 10% heat-inactivated fetal calf serum (HyClone Laboratories, Inc., Logan, Utah), and 5×10^{-5} M 2-mercaptoethanol (Wako Pure Chemical, Osaka, Japan). After 100 μl of each sample was added to 3 wells in a 96-well plate (Corning Glass Works, Corning, N.Y.), 100 μl of the cell suspension was mixed and cultured at 37°C for 2 days under 5% CO_2 gas. Four hours before the termination of incubation, 10 μl of a solution containing 5 mg of MTT (Sigma Chemical, St. Louis, Mo.) per ml was added. After incubation, plates were centrifuged at 400 × *g* and 4°C for 10 min, and the supernatants were aspirated. The reduction of MTT by the mitochondrial dehydrogenases of viable cells yielded blue formazan crystals at the bottom of the plate. One hundred microliters of dimethyl sulfoxide was added to the cell sediment to solubilize the formazan crystals. After the plate was agitated on a plate mixer (Taiyo Chemical Industrial Co., Ltd., Tokyo, Japan), the optical densities at 550 to 660 nm were determined with an enzyme-linked immunosorbent assay analyzer (ETY-96; Oriental Instruments Ltd., Tokyo, Japan). The cell proliferation rate was expressed as the ratio (in percent) of formazan formation in treated cells to that in the control cells incubated without antibiotics.

Statistical analysis. The significance of differences from the preexposure sample was analyzed by Student's *t* test.

RESULTS

Spectral changes of compounds differing at the 8 position by UVA irradiation. When aqueous solutions of Q-35 (8-OCH_3) and its 8-H and 8-F analogs at 20 μg/ml were irradiated with 3 J of UVA per cm^2, spectral changes were observed (Fig. 1). The maximal absorption of Q-35 (8-OCH_3) was at 333 and 286 nm, and no alteration of the absorbance spectrum was detected after irradiation. The maximal absorption of the 8-F analog was at 328 and 282 nm, but it shifted to 273 nm after irradiation. Similarly, the absorption spectrum of the 8-H analog was also markedly changed by irradiation with UVA.

Quantitation of undegraded compounds. Aqueous solutions treated as described above were submitted to high-performance liquid chromatography (HPLC) for the quantitation of undegraded compounds. Q-35 (8-OCH_3) remained almost intact after UVA irradiation of 3 J/cm^2, and 83.1% ± 3.52% of the compound was left unchanged even after it received UVA irradiation at 10 J/cm^2 (Fig. 2). In contrast, UVA irradiation degraded the 8-H analog to 57.8% ± 3.67% with irradiation at 0.3 J/cm^2, 15.9% ± 4.16% with irradiation at 1 J/cm^2, and below 1% with irradiation at 3 J/cm^2. Irradiation at 0.3 J/cm^2 degraded the 8-F analog to below 1% of its original form. After exposure to increasing doses of UVA, a number of photoproducts of the 8-H and 8-F analogs were observed upon HPLC analysis (data not shown).

Alteration of antibacterial activity. Similarly, the antibacterial activities of aqueous solutions irradiated with UVA were compared. The antibacterial activity of Q-35 (8-OCH_3) was not altered when it was exposed to up to 10 J of irradiation per cm^2 (Table 1). In contrast, the antibacterial activity of the 8-F analog began to decrease with UVA irradiation from 1 J/cm^2, and 20% of the activity remained after 10 J of irradiation per cm^2. In addition, the antibacterial activity of the 8-H analog was halved after 1 J of UVA

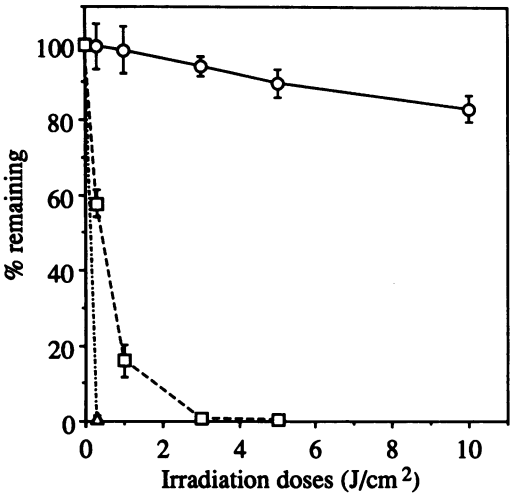

FIG. 2. Effect of UVA irradiation on the stability of 8-substituted fluoroquinolones. The amounts of undegraded Q-35 (8-OCH_3; ○), the 8-F analog (△), and the 8-H analog (□) with increasing doses of UVA were determined by HPLC.

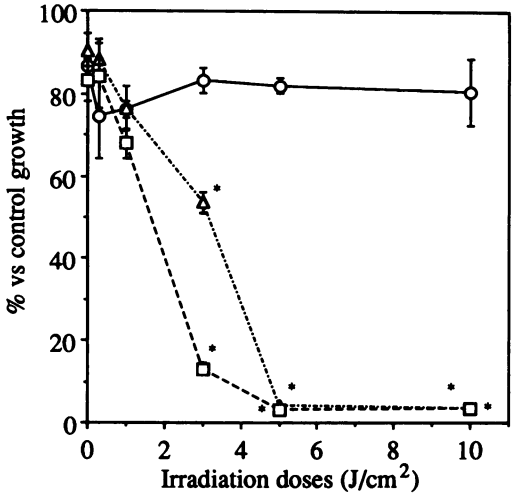

FIG. 3. Cytotoxicities of UVA-irradiated solutions against L-1210 cells. The leukemic cells were incubated with the irradiated solutions of Q-35 (8-OCH_3; ○), the 8-F analog (△), and the 8-H analog (□) at 37°C for 2 days. The viability was measured by the MTT method. *, $P < 0.001$ versus the cytotoxicity of the preexposure solution.

irradiation per cm^2 and less than 10% remained after irradiation at above 5 J/cm^2.

Change in cytotoxicity. The preexposure proliferation inhibitory activities of Q-35 (8-OCH_3) and its 8-H and 8-F analogs at concentrations of 20 μg/ml were all about 10% (Fig. 3), and the activities were not significantly different among these three compounds. The cytotoxicity of Q-35 (8-OCH_3) did not change from preexposure to postexposure. On the other hand, the 8-H and 8-F analogs of Q-35 significantly inhibited cell proliferation after they received 3 J of irradiation per cm^2 and completely inhibited the proliferation after they received 5 J of irradiation per cm^2.

TABLE 1. Anti-*B. subtilis* activity of UVA-irradiated solutions

8-Substituted fluoroquinolone	Irradiation dose (J/cm^2)	Zone diam (mm)[a]	Residual activity (%)[a,b]
Q-35 (8-OCH_3)	0	30.5 ± 0.25	
	0.3	30.2 ± 0.13	96.8 ± 3.83
	1	30.0 ± 0.30	92.9 ± 8.57
	3	30.5 ± 0.09	105 ± 3.18
	5	30.1 ± 0.25	94.5 ± 7.61
	10	29.5 ± 0.53	82.8 ± 14.1
8-F analog	0	29.7 ± 0.42	
	0.3	27.7 ± 0.66	54.7 ± 11.0
	1	25.6 ± 0.17[c]	32.1 ± 1.69
	3	24.9 ± 0.37[c]	26.5 ± 3.03
	5	24.1 ± 0.25[c]	21.5 ± 1.70
	10	24.2 ± 0.09[c]	22.4 ± 0.66
8-H analog	0	24.1 ± 0.36	
	0.3	23.8 ± 0.65	87.0 ± 17.4
	1	21.2 ± 0.45	46.4 ± 5.94
	3	17.9 ± 0.28[c]	20.7 ± 1.69
	5	14.4 ± 0.33[c]	8.95 ± 0.86
	10	9.90 ± 0.09[c]	2.96 ± 0.08

[a] Values are means ± standard deviations for three disks.
[b] Antibacterial activity was determined from the standard curves, which were made with increasing concentrations of each compound.
[c] $P < 0.001$ versus the antibacterial activity of the preexposure solution.

Effect of UVA irradiation on known fluoroquinolones. Changes in the absorption spectra of known fluoroquinolones by UVA are shown in Fig. 4. LFLX with fluoride at the 8 position and ENX with a naphthyridine ring underwent spectral changes after they received 0.3 J of UVA irradiation per cm^2. The spectral changes in NFLX, CPFX, and OFLX were initiated by 1 J of irradiation per cm^2, and these changes became more prominent with 3 J of irradiation per cm^2. The decreases in antibacterial activities and increases in cytotoxicities corresponded to the spectral changes (data not shown).

DISCUSSION

Some of the fluoroquinolone antibiotics that are used clinically are unstable to irradiation with UV light, including natural sunlight, and undergo photodegradation. Indeed, LFLX and CPFX are reported to be photodegraded by sunlight and UV light at about 320 nm, respectively, and are reported to lose their antibacterial activities after exposure to UV light (10, 16, 20).

In the study described here, when 8-F, an analog of Q-35 substituted by fluorine at the 8 position, and 8-H, the unsubstituted analog of Q-35, in aqueous solution were irradiated at doses ranging from 0.3 to 3 J of UVA per cm^2, the parent compounds disappeared, as detected by HPLC, and there was a concomitant loss of antibacterial activity that accompanied an increase in cytotoxicity. These observations could not be made with Q-35 (8-OCH_3) substituted at the 8 position with a methoxy group. Similarly, UVA irradiation above 0.3 to 1 J/cm^2 induced spectral changes in fluoroquinolone antibiotics that are on the market. The instability did not differ between NFLX and CPFX, compounds which are substituted at the 1 position by ethyl and cyclopropyl groups, respectively; but ENX, with a naphthyridine ring, was found to undergo more profound alterations at a low dose of irradiation than NFLX, which has a quinolone ring. UVA (320 to 400 nm) has potent skin-penetrating activity and elicits most of the known phototoxic

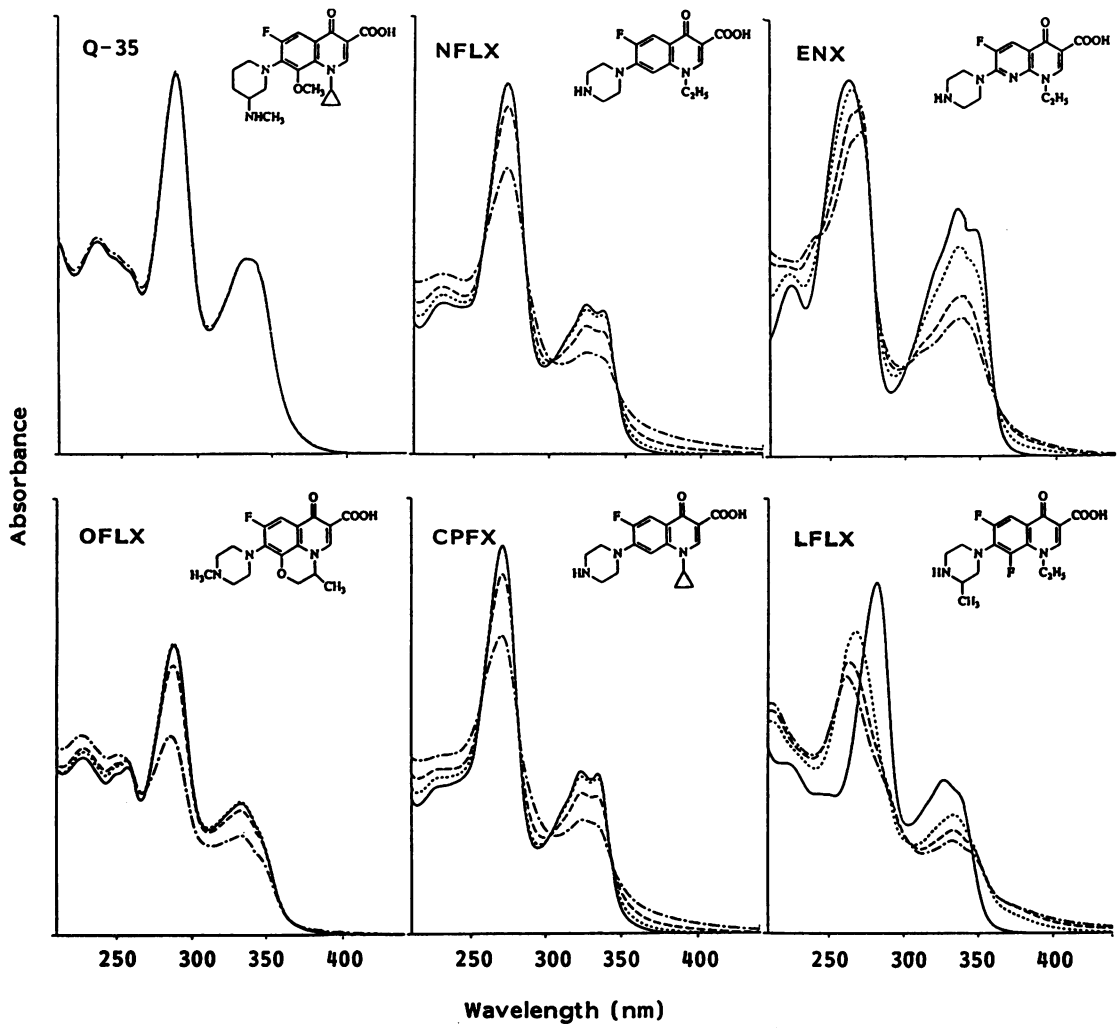

FIG. 4. Effect of UVA irradiation on known fluoroquinolones. A solution containing 20 μg of a fluoroquinolone per ml was irradiated with 0 (——), 0.3 (····), 1 (– – –), or 3 (– · – · –) J of UVA per cm^2 under the same conditions described in the legend to Fig. 1.

and photoallergic chemical reactions. UVA at a dose of 2 to 3 J/cm^2 per h corresponds to that of daytime sunlight in late fall. Therefore, a similar phenomenon might take place on the surfaces of human skins that can be reached by UVA.

As a matter of fact, NA is clinically known as a photosensitizer (1, 3, 4), and the photosensitivities of some of fluoroquinolone antibiotics that are on the market have also been reported (2, 5, 13, 14). In order to compare the phototoxicity potentials of these quinolone antibiotics, Wagai et al. (21) investigated UVA irradiation (21.6-J/cm^2)-induced erythema as a biological marker; the erythema occurs in the ears of mice after oral administration of NA. All mice that were administered 200 mg of NA per kg of body weight had erythema in the ears between the end of irradiation and 24 h postexposure, and the dose for 50% erythema induction was 143 mg/kg. Similar phototoxicities were detected with other fluoroquinolone antibiotics, and their potencies were LFLX > ENX, NA > OFLX, DR-3355 > CPFX, in that order. The phototoxicity of Q-35 (8-OCH_3) has so far not been observed under the conditions of erythema induction in mice described above. This might be attributed to its in vitro stability against UVA irradiation. Therefore, the 8-F and 8-H analogs, like LFLX and CPFX, may provoke phototoxicity in vivo.

Drug-induced photosensitivity is generally initiated by the absorption of light energy into drugs and is then provoked by consequent chemical reactions in the body. Investigators have determined three possible reaction mechanisms in studies of phototoxic reactions (7). The first possibility is that activated drugs directly bind covalently to DNA and proteins. The second possibility is that the stable photoproducts that are formed cause injury to the body. The third possibility is that drugs transfer their absorbed energy to oxygen molecules, thus forming reactive oxygen, which nonspecifically injures the body. Experiments with in vitro photohemolysis of NA have suggested the third possibility (11). Photoproducts of NA promote photohemolysis but are distinct from chlorpromazine in that they do not induce hemolysis of erythrocytes without light. Ferguson et al. (8, 9) have reported that while CPFX, which is different from NA, does not induce photohemolysis, both compounds promote the photosensitivity of macrophages and phytohemagglutinin-stimulated lymphocytes. From these results, they assumed that while membrane-dependent reactions would

play a major role in NA phototoxicity, CPFX-induced phototoxicity would be mediated more through an intracellular target. However, they could not obtain evidence on the formation of toxic photoproducts with NA and CPFX. In this report, we suggest the existence of fluoroquinolone photoproducts, with cytotoxicity potentiated by photoreactions. These photoreactions demonstrated an increased cytotoxicity with the simultaneous disappearance of the undegraded compounds. However, the relation of the increased cytotoxicity and decreased antibacterial activity to the photodegradation of the 8-H analog is different from their relation to photodegradation of the 8-F analog. While the changes in the biological activities of the 8-H analog were closely associated with degradation, those of the 8-F analog occurred later than degradation did. The reason for these differences remains unclear, and it will remain so until the UV degradation products of the 8-H and 8-F analogs have been identified. In terms of the disappearance of the undegraded compound with increasing doses of UVA, LFLX and ENX are more sensitive than NFLX, CPFX, and OFLX. These results seem to relate to the phototoxicity test in mice. Therefore, the instability against UVA, a physicochemical property of fluoroquinolones, appears to be closely associated with the potential for photosensitivity. However, the relationship between the instability of a drug to UVA and the changes in biological activities is not clear and needs to be investigated further.

From the results presented here, the introduction of a methoxy group at the 8 position of the quinolone ring plays an important role in stabilizing fluoroquinolone antibiotics against UVA irradiation. At present, fluoroquinolone antibiotics are widely used against infectious diseases of the respiratory organ, urinary tract, and skin because of their excellent antibacterial activities and distributions in tissues. We are interested in how stabilization of fluoroquinolone antibiotics against UVA, which is permeable through the skin (as shown in this report), can be attributed not only to their efficacies but also to decreases in their local and general side effects caused by photosensitivity. Additional studies are required for elucidating the relationship between the in vitro instability against UV irradiation and the in vivo phototoxicities of the 8-H and 8-F analogs of the fluoroquinolone antibiotic Q-35. It is expected that those studies will reveal that the phototoxic mechanism is the cause of the abnormal photosensitivities of fluoroquinolones.

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REFERENCES

1. **Baes, H.** 1968. Photosensitivity caused by nalidixic acid. Dermatologica **136**:61–64.
2. **Baran, R., and P. Brun.** 1986. Photoonycholysis induced by the fluoroquinolones pefloxacin and ofloxacin: report on 2 cases. Dermatologica **173**:185–188.
3. **Birkett, D. A., M. Garretts, and C. J. Stevenson.** 1969. Phototoxic bullous eruptions due to nalidixic acid. Br. J. Dermatol. **81**:342–344.
4. **Boisvert, A., and G. Barbeau.** 1981. Nalidixic acid-induced photodermatitis after minimal sun exposure. Drug Intell. Clin. Pharm. **15**:126–127.
5. **Bowie, W. R., V. Willetts, and P. J. Jewesson.** 1989. Adverse reactions in a dose-ranging study with a new long-acting fluoroquinolone, fleroxacin. Antimicrob. Agents Chemother. **33**: 1778–1782.
6. **Christ, W., T. Lehnert, and B. Ulbrich.** 1988. Specific toxicologic aspects of the quinolones. Rev. Infect. Dis. **10**(Suppl. 1):S141–S146.
7. **Epstein, J. H., and B. U. Wintroub.** 1985. Photosensitivity due to drugs. Drugs **30**:42–57.
8. **Ferguson, J., and B. E. Johnson.** 1990. Ciprofloxacin-induced photosensitivity: in vitro and in vivo studies. Br. J. Dermatol. **123**:9–20.
9. **Ferguson, J., J. McIntosh, and E. M. Walker.** 1988. Ciprofloxacin-induced photosensitivity: in vitro and in vivo studies. J. Invest. Dermatol. **91**:385.
10. **Ferguson, J., G. Philips, and J. McEwen.** 1988. Loss of antibiotic activity caused by photodegradation: in vitro studies. Br. J. Dermatol. **119**:550–551.
11. **Fernandez, E., A. M. Cardenas, and G. Martinez.** 1987. Phototoxicity from nalidixic acid: oxygen dependent photohemolysis. Farmaco (Sci.) **42**:681–690.
12. **Halkin, H.** 1988. Adverse effects of the fluoroquinolones. Rev. Infect. Dis. **10**(Suppl. 1):S258–S261.
13. **Jensen, T., S. S. Pedersen, and C. H. Nielsen.** 1987. The efficacy and safety of ciprofloxacin and ofloxacin in chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. J. Antimicrob. Chemother. **20**:585–594.
14. **Kawabe, Y., N. Mizuno, and S. Sakakibara.** 1989. Photoallergic reactions caused by enoxacin. Photodermatology **6**:58–60.
15. **Kojima, K., S. Matsubara, M. Matsumoto, T. Ito, A. Kondo, and T. Yokota.** 1991. In vitro activity of Q-35, a new 8-methoxy quinolone. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1444.
16. **Leigh, D. A., S. Tait, and B. Walsh.** 1991. Antibacterial activity of lomefloxacin. J. Antimicrob. Chemother. **27**:589–598.
17. **Matsubara, S., M. Matsumoto, M. Ishigai, M. Nakagami, Y. Nabuchi, F. Takahashi, and K. Tanaka.** 1991. Pharmacokinetics and pharmacology of Q-35, a new 8-methoxy quinolone, in experimental animals. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1445.
18. **Mosmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J. Immunol. Methods **65**:55–63.
19. **Nagano, H., T. Yokota, and Y. Katoh.** Sept. 1991. Novel quinolonecarboxylic acid derivatives. U.S. patent 5,051,509.
20. **Phillips, G., B. E. Johnson, and J. Ferguson.** 1990. The loss of antibiotic activity of ciprofloxacin by photodegradation. J. Antimicrob. Chemother. **26**:783–789.
21. **Wagai, N., F. Yamaguchi, M. Sekiguchi, and K. Tawara.** 1990. Phototoxic potential of quinolone antibacterial agents in Balb/c mice. Toxicol. Lett. **54**:299–308.