New Findings on the Structure-Phototoxicity Relationship and Photostability of Fluoroquinolones with Various Substituents at Position 1

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Fluoroquinolones (FQs) are very effective for the treatment of various bacterial infections. The results of studies of the structure-activity relationships of FQs have been reported previously (5, 19). It has also been demonstrated that a cyclopropyl group at position 1, in combination with a 3-aminopyrrolidinyl group at position 7 and a halogen atom such as fluorine or chlorine at position 8, confers the most potent antibacterial activity on the FQ molecule. In particular, the introduction of a halogen atom at position 8 provides an FQ molecule with potent antibacterial activity in vivo via expansion of the antibacterial spectrum and improvements in oral bioavailability.

Clinical and experimental studies of most FQs developed in the past have reported that they cause phototoxicity with various degrees of severity (2, 3, 5, 9, 10, 18, 19). Derivatives of this class of drugs containing a halogen atom at position 8 were found to have the greatest phototoxic properties. Lomefloxacin, sparflaxin, floxacin, and clinafloxacin are included in this group of FQs. On the other hand, a halogen group at position 8 provides an FQ molecule with potent antibacterial activity in vivo via expansion of the antibacterial spectrum and improvements in oral bioavailability.

Clinical and experimental studies of FQs have examined the phototoxicities of a series of 7-(3-aminopyrrolidinyl) quinolones containing various substituents at position 1 (in which the substituent at R8 is a hydroxyl or a halogen) by use of a mouse model. The results of studies of the structure-activity relationships of FQs have been reported previously (5, 19). It has also been demonstrated that a cyclopropyl group at position 1, in combination with a 3-aminopyrrolidinyl group at position 7 and a halogen atom such as fluorine or chlorine at position 8, confers the most potent antibacterial activity on the FQ molecule. In particular, the introduction of a halogen atom at position 8 provides an FQ molecule with potent antibacterial activity in vivo via expansion of the antibacterial spectrum and improvements in oral bioavailability.

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groups, were used. We have reported that these novel substituent groups offer a broad antibacterial spectrum and excellent antibacterial activity against gram-positive and gram-negative pathogens. We established the protective effect of a 1-aminodifluorophenyl group against phototoxicity using structurally similar pairs of compounds with practically the same areas under the concentration-time curves from 0 to 4 h (AUC0-4s) in tissue. We also investigated the effect of this N-1 substituent on photodegradation, since observation of the photodegradation process would give us clues to help us understand the initial stage of phototoxicity.

MATERIALS AND METHODS

Compounds. The reference FQs (Fig. 1) and various derivatives (see Table 3) used in this study were synthesized in our laboratory.

Animals. Female ICR strain mice (age, 5 to 6 weeks; weight, 23 to 32 g; Charles River Japan Inc., Hino, Japan) were used in this study. They were housed in plastic cages in groups of six and were maintained in an air-conditioned room (temperature, 23 ± 2 °C; relative humidity, 55 ± 12%) with free access to commercial laboratory chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and tap water.

UVA irradiation system. A UV type A (UVA) irradiation system was used to provide irradiation, as described by Marutani et al. (15). Briefly, the source of UVA light consisted of a bank of 10 black-light fluorescent bulbs (FL20SBLB; Toshiba, Tokyo, Japan) that emit radiation over a wavelength range of 300 to 400 nm. A 3-mm-thick glass plate was placed over a multichamber box (in which partitions divide the box into two by six chambers) to absorb light at wavelengths below 320 nm (UVB). The radiation dose was delivered at a distance of 15 cm from the light and was 1.8 mW/s/cm2 in the irradiated region, as measured with a digital illuminometer with interchangeable sensors (DRC-100X; Spectronics Corporation, Westbury, N.Y.). An electric fan was placed 50 cm away from the light source to prevent an increase in temperature in the irradiated area.

Phototoxicity test. Phototoxicity tests were performed by the methods described by Marutani et al. (15) and Wagai et al. (21, 22), with some modifications. Briefly, ICR mice received intravenous doses of FQs (40 mg/10 ml/kg) and were then placed into the chambers of the multichamber box and exposed to UVA light for 4 h (26 J/cm2), with a 15-cm distance between the lights and the ears of the mice. The FQs were dissolved in 0.1 N NaOH. Control mice that received 0.1 N NaOH were also irradiated. The reddness of the ears of the mice was used as a phototoxicity index and was scored as follows at 0, 24, 48, 72, and 168 h after the irradiation: 0, normal; 1, mild erythema; 2, moderate erythema; 3, severe erythema and edema formation.

AUC measurements. ICR mice were given a single intravenous dose of one of the FQs. For each of the time points of 15 and 30 min and 1, 2, and 4 h after drug administration, four mice were anesthetized with ether and the blood and the ears were collected. The blood samples were centrifuged to obtain serum. The ears were added to 1 N KOH and were maintained at 60 °C for 1 h, following neutralization with 0.8 M phosphoric acid. The concentrations of FQs in the serum and ears were quantified with a high-performance liquid chromatograph (LC-10A; Shimadzu, Tokyo, Japan) with a TSK gel ODS-80Ts column. A mixture of 20 mM sodium dodecyl sulfate-Pi and acetonitrile (57:33; vol/vol) was used as the mobile phase at a flow rate of 1.0 ml/min. The column eluent was monitored at 290 nm. The AUC0-4s of the FQs were calculated by the trapezoid method.

Photostability. The FQs were dissolved in 0.02 N NaOH to a concentration of 2 mg/ml and were then diluted with 0.1 M phosphate buffer (pH 7.0) to achieve a final concentration of 20 µg/ml. Each of these solutions was transferred to four wells of a 24-well plate (1 ml/well); and the plates were irradiated with UVA light (1.8 mW/cm2) for 2, 5, 10, and 20 min, respectively. The control solution was maintained in the dark. To examine the effect of excess chloride ions on the photodegradation of FQs, 0.1 M phosphate buffer (pH 7.0) containing 0.5 M NaCl was used. The residual amounts of FQs were quantified with a high-performance liquid chromatograph (LC-10AT, Shimadzu) with a TSK gel ODS-80Ts column. A mixture of 20 mM sodium dodecyl sulfate-Pi and acetonitrile (3:2) was used as the mobile phase at a flow rate of 1.2 ml/min. The column eluent was monitored at 254 nm.
RESULTS

Dose-response study of phototoxicities of reference quinolones in a mouse model. To determine the proper dose and observation intervals for screening of the phototoxic potentials of the FQs, the dose-response relationships and the time course of toxicity were examined with levofloxacin, tosufloxacin, and lomefloxacin, which are known from experimental and clinical studies (2, 4, 5, 10, 11, 19) to be mildly, moderately, and severely phototoxic, respectively. These FQs were administered to ICR mice as an intravenous bolus to avoid the bias caused by the diversity of murine intestinal absorption of FQs. Immediately after FQ administration, the mice were exposed to UVA light for 4 h. Ear erythema was observed and was classified by use of the phototoxicity index for up to 168 h after UVA irradiation.

As shown in Table 1, we could determine whether the ear erythema was aggravated or alleviated when we observed the ears 0 and 48 h after UV irradiation. We were able to evaluate the phototoxic potencies of all the quinolones when they were used at a dose of 40 mg/kg.

Phototoxicities of reference quinolones. Next, the phototoxic potentials of the reference quinolones (Fig. 1) at a dose of 40 mg/kg were examined in the mouse model. The reference quinolones were categorized into four classes: 8-halogeno quinolones, such as lomefloxacin, fleroxacin, clinafloxacin, and norfloxacin, with a 1-cyclopropyl or a 1-ethyl group were as severe as those of the well-known substituent at position 1. Additionally, an aminodiaryl fluorophenyl, an isoxazolyl, or an oxetanyl group was used as novel substituents. Compounds with these groups offered broad-spectrum and excellent activity against gram-positive and gram-negative pathogens (data not shown).

Unexpectedly, the phototoxicities of the 8-H quinolones with a 1-cyclopropyl or a 1-ethyl group were as severe as those of the reference 8-halogeno quinolones, such as lomefloxacin, fleroxacin, clinafloxacin, and norfloxacin (Table 2). On the contrary, the 8-H quinolones with a 1-difluorophenyl or a 1-oxetanyl group were moderately phototoxic; 8-H quinolones with a 1-aminodifluorophenyl or a 1-isoxazolyl group were mildly phototoxic. Surprisingly, despite the substitution of a halogen atom at position 8, the quinolones with a 1-aminodifluorophenyl or a 1-isoxazolyl group caused mild phototoxicity. This is the first finding of mildly phototoxically 8-halogeno quinolones. This mild phototoxicity might have been due to the pharmacokinetic instability of these quinolones. To examine this possibility, we measured the AUC0-4s of a pair of structurally similar quinolones in serum and auricular tissue: the 1-amino- difluorophenyl 8-chloro quinoline (which was mildly phototoxic) and the 1-difluorophenyl 8-chloro quinoline (which was severely phototoxic). The chemical structures of both quinolones were identical except for the amino group at position 1.
Distributions of 8-halogeno quinolones into the ears of mice. The concentrations of the quinolones with a 1-difluorophenyl or a 1-aminodifluorophenyl 8-chloro group in the sera and ear tissues of mice were determined after intravenous administration of the quinolones (40 mg/kg; \( n = 4 \)). As shown in Fig. 2A and B, the pharmacokinetic profiles of both compounds were similar. The \( \text{AUC}_{0-4} \) of the 1-difluorophenyl quinolone and the 1-aminodifluorophenyl quinolone were 18.2 and 16.7 \( \mu \text{g} \cdot \text{h/g} \), respectively, in the ears and 22.5 and 26.3 \( \mu \text{g} \cdot \text{h/ml} \), respectively, in sera, indicating that the mildly phototoxic potential of the 1-aminodifluorophenyl quinolone was not due to its poor distribution into the ears.

Photostabilities of 8-halogeno quinolones. The photostability studies were carried out with the pair of 8-chloro quinolones mentioned above. Chloride ions inhibited the photodegradation of the 1-difluorophenyl 8-chloro quinolone (Fig. 3A), as reported previously for an 8-chloro quinolone (1). However, the effect of the chloride ions was not observed with the 1-aminodifluorophenyl 8-chloro quinolone (Fig. 3B).

### DISCUSSION

In this study, the phototoxic potentials of newly synthesized FQs with various substituents at position 1 were determined systematically. The most important new conclusion from this study is that the phototoxic potentials of FQs are affected not only by the substituent at position 8 but also by that at position 1.

Several clinical and experimental studies have reported that FQs with a halogen atom at position 8 cause severe phototoxicity (2, 5, 9, 13, 15, 18, 19), while 8-hydrogen quinolones cause much milder phototoxicity (5, 8, 9, 19, 20). On the contrary, the 8-methoxy quinolones did not cause any significant phototoxicity (12, 13, 15). In the present study, we examined the structure-phototoxicity relationships of a series of 7-(3-aminopyrrolidinyl) quinolones with various substituents at position 1 (in which \( R_8 \) contained a hydrogen or halogen) using a mouse model. The degree of phototoxicity of FQs with a difluorophenyl group at position 1 changed from mild to severe when a hydrogen atom was replaced by a halogen at position 8. On the other hand, the 1-cyclopropyl or the 1-ethyl quinolones were severely phototoxic, regardless of the substituents at position 8, and caused severe phototoxicity comparable to those of the reference 8-halogeno quinolones. Furthermore, the 1-aminodifluorophenyl and the 1-isoxazolyl quinolones caused mild

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\(^a\) Each compound was administered at a dose of 40 mg/kg.

\(^b\) The vehicle was 0.1 N NaOH.

\(^c\) The scores for ear redness were as follows: 0, normal; 1, mild erythema; 2, moderate erythema; 3, severe erythema and edema.

![FIG. 2. Pharmacokinetics of the 1-difluorophenyl 8-chloro quinolone and the 1-aminodifluorophenyl 8-chloro quinolone in the sera (A) and ears (B) of mice to which the FQs were administered intravenously (40 mg/kg).](image)

![FIG. 3. Photodegradation of the 1-difluorophenyl 8-chloro quinolone (A) and the 1-aminodifluorophenyl 8-chloro quinolone (B) in 0.1 M phosphate buffer in the presence or absence of excess chloride ions.](image)
photic activity immediately after irradiation, which disappeared by 48 h, despite the substitution of a halogen atom at position 8. These results indicate that the phototoxic potentials of FQs are influenced not only by the substituent at position 8 but also by that at position 1.

It is well known that the substitution of a halogen atom at position 8 expands the spectrum of antibacterial activity and improves the oral bioavailability (5). However, there are some problems with 8-methoxy quinolones, which clinical studies have shown is a safe fluoroquinolone. In conclusion, the phototoxic potentials of FQs were influenced not only by the substituent at position 8 but also by that at position 1.

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REFERENCES