Photochemical Behavior of Sitafloxacin, Fluoroquinolone Antibiotic, in an Aqueous Solution

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STFX hydrate, an antimicrobial agent, is photo-labile in aqueous solutions. The photodegradation rates (k) in neutral solutions were higher than those observed in acidic and alkaline solutions and maximum at the maximum absorption wavelength of STFX. The structures of photodegradation products were elucidated as 7-[7-amino-5-azaspiro[2.4]heptan-5-yl]-8-chloro-6-fluoro-1-cyclopropyl]-1,4-diacyl-4-oxo-3-quinolinecarboxylic acid sesquihydrate and 7-[7-amino-5-azaspiro[2.4]heptan-5-yl]-8-bromo-6-fluoro-1-cyclopropyl]-1,4-diacyl-4-oxo-3-quinolinecarboxylic acid sesquihydrate. These imply that chlorination is the key step in the photodegradation of STFX. The effect of halide ions on the photodegradation of STFX was estimated by observing the increments in the photostability of STFX with the addition of chloride ions. In contrast, in the presence of bromide ions, instead of increased photostability of the STFX rate, a new photodegradation product in the presence of bromide ion was observed. The structure of this new photodegradation product was an 8-bromo form of STFX, which was substituted for chlorine at the 8-position, so the dissociation of C–Cl bond at the 8-position of STFX was the rate-limiting step in the initial process of the photodegradation. STFX generated ·C (carbon centered radical) and ·OH (hydroxyl radical) in the process of photodegradation in a pH 4.0 buffer. On the contrary, STFX did not generate ·C in the presence of chloride ion in a pH 4.0 buffer. The · C was generated and then degraded into the above degradation products by photolysis in the absence of chloride ion, but the ·C immediately reacted with chloride when it was present. As a result, the C–Cl bond was recovered leading to a possible increase in the apparent photostability.

Key words antimicrobial; fluoroquinolone; sitafloxacin; photodegradation

STFX was synthesized at Daiichi Pharmaceutical Co., Ltd., 16–13 Kita-Kasai 1-chome, Edogawa-ku, Tokyo 134–8630, Japan. (Tokyo) at approximately 10000 lux for ca. 350000 lux·h. After irradiation, the solution was loaded onto a preparative HPLC system and fractionated.

Preparative HPLC was carried out on a gradient system consisting of an LC 10AD pump (Shimadzu Co., Kyoto) equipped with a SPD-6A UV/vis detector (Shimadzu Co.). HPLC conditions were as follows: column, TSKgel ODS-80TM (21.5 mm i.d.×300 mm, Tosoh Co., Tokyo); eluent, 0.05 m phosphate buffer (pH 2.4): acetonitrile=82:18 (from 0 to 65 min) to 50:50 (from 65 to 100 min); flow rate, 5.0 ml/min (from 0 to 65 min) to 7.0 ml/min (from 65 to 100 min); injection, 2 ml; detection, 278 nm; column temperature, 40°C. The collected fractions were concentrated and lyophilized. Then each sample was dissolved in water and loaded onto a desalting preparative HPLC. HPLC conditions were as follows: column, TSKgel ODS-80TM (21.5 mm i.d.×300 mm, Tosoh Co.); eluent, 0.1% acetic acid and acetonitrile=30:70; flow rate, 7.0 ml/min; injection, 2 ml; detection, 278 nm; column temperature, 40°C. Isolated photodegradation products were elucidated by using a 1H-NMR (JNM-GSX500 FT-NMR spectrometer (500 MHz), JEOL Co., Tokyo) and electron impact (EI), chemical ionization (CI), fast atom bombardment (FAB)-MS (JMS-HX110 mass spectrometer, JEOL Co.) spectra. Kinetics of Photodegradation STFX was dissolved in Britton–Robin-son buffer solutions of pH 2.0–11.0 and 0.1 mol/l sodium hydroxide (pH 13.0) (STFX, about 50 µg/ml). Each sample solution (2 ml) was poured into a glass container (internal diameter: ca. 20 mm), which was covered with clear polyvinyl chloride film. Then, an exposure test was performed at 25°C by irradiation with a D65 fluorescent lamp; this is an artificial daylight fluorescent lamp that emits visible and ultraviolet lights. The illumination of the D65 fluorescent lamp was adjusted to 4000 lux; at this illumination the near ultraviolet energy was about 120 µW/cm². At appropriate time intervals, the sample solution was withdrawn, mixed with the HPLC mobile phase, and the resulting solution (ca. 5 µg/ml) was assayed by HPLC.

Fig. 1. Chemical Structure of STFX Hydrate

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Materials STFX was synthesized at Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.

Structural Elucidation of Photodegradation Products Ninety-four milligrams of STFX hydrate was dissolved in 1 l of purified water and irradiated with fluorescent lamps (Biophotochamber LX-2100 (TAITEC Co., Tokyo)) at approximately 10000 lux for ca. 350000 lux·h. After irradiation, the solution was loaded onto a preparative HPLC system and fractionated.

Preparative HPLC was carried out on a gradient system consisting of an LC 10AD pump (Shimadzu Co., Kyoto) equipped with a SPD-6A UV/vis detector (Shimadzu Co.). HPLC conditions were as follows: column, TSKgel ODS-80TM (21.5 mm i.d.×300 mm, Tosoh Co., Tokyo); eluent, 0.05 m phosphate buffer (pH 2.4): acetonitrile=82:18 (from 0 to 65 min) to 50:50 (from 65 to 100 min); flow rate, 5.0 ml/min (from 0 to 65 min) to 7.0 ml/min (from 65 to 100 min); injection, 2 ml; detection, 278 nm; column temperature, 40°C. The collected fractions were concentrated and lyophilized. Then each sample solution (2 ml) was poured into a glass container (internal diameter: ca. 20 mm), which was covered with clear polyvinyl chloride film. Then, an exposure test was performed at 25°C by irradiation with a D65 fluorescent lamp; this is an artificial daylight fluorescent lamp that emits visible and ultraviolet lights. The illumination of the D65 fluorescent lamp was adjusted to 4000 lux; at this illumination the near ultraviolet energy was about 120 µW/cm². At appropriate time intervals, the sample solution was withdrawn, mixed with the HPLC mobile phase, and the resulting solution (ca. 5 µg/ml) was assayed by HPLC.

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Wavelength Dependency of Photodegradation

Three milliliters of aqueous solution of STFX (500 µM, pH 7.4 phosphate buffer) in a quartz glass cell was irradiated for 0—10 J/cm² at various wavelengths (699, 649, 599, 499, 446, 419, 392, 365, 338, 311, 284, 257, 230 nm) by a grating monochromator apparatus, CRM-FA equipped with a Xe lamp (Japan Spectroscopic Co., Tokyo).

HPLC conditions were as follows: column, TSKgel ODS-80Ts (4.6 mm i.d.×150 mm, Tosoh Co.); eluent, 0.05M phosphate buffer (pH 2.4); acetonitrile=80:20 (from 0 to 10 min), 36:64 (from 10 to 20 min), 80:20 (from 20 to 40 min); flow rate, 1.0 ml/min; injection, 20 µl; detection, 290 nm; column temperature, 40°C.

Liquid Chromatography (LC)-MS Analysis of a Photodegradation Product of STFX in the Presence of Bromide Ions

STFX was dissolved in 0.05M citrate buffer (pH 4.0) containing 0.2M NaBr, and the resulting solution (about 2mg/ml) was exposed to light from a D65 fluorescent lamp, which had an overall illumination of ca. 368000lux·h. After the reaction, the sample solution was mixed with the HPLC mobile phase and the resulting solution (ca. 50µg/ml) was subjected to LC-MS, the conditions of which were as follows: column, YMC AM-302, 4.6 mm i.d.×150 mm; eluent, trifluoroacetic acid (TFA) aqueous solution (pH 2.4)-acetonitrile (73:27, v:v); flow rate, 0.2 ml/min; detection, UV 294 nm; column temperature, 40°C. Electrospray ionization (ESI)-MS conditions of which were as follows: ion mode, positive; spray kilovolts, 4.5 kV; heated capillary, 225°C; sheath gas (N2), 80 psi; auxiliary gas (N2), 10 units.

Effect of Chloride Ion on the Photodegradation Kinetics of STFX

STFX was dissolved in 0.05M citrate buffer (pH 4.0) containing 0.2M NaCl or 0.2M of NaBr (STFX, about 50µg/ml). Each solution (2ml) was poured into a glass container (internal diameter: ca. 20mm), which was covered with clear polyvinylidene chloride film. Then, an exposure test was performed at 25°C by irradiating these sample solutions with Light-Tron LF-120. The illumination of the D65 fluorescent lamp was adjusted to 4000 lux; at this illumination the near ultraviolet energy was about 120µW/cm². At appropriate time intervals, the sample solution was withdrawn, mixed with the HPLC mobile phase, and the resulting solution (ca. 5µg/ml) was assayed by HPLC. The HPLC conditions were as follows: column, TSKgel ODS-80Ts (4.6 mm i.d.×250 mm, Tosoh Co.); eluent, 0.05M phosphate buffer (pH 2.4)-acetonitrile (70:30, v:v) containing 0.02M ammonium acetate and 5mM sodium 1-nonen sulfonate; flow rate, 0.8 ml/min; injection, 50 µl; detection, UV 294 nm; column temperature, 40°C.

Determination of Acid Dissociation Constants (pKa) of STFX

The acid dissociation constants of STFX were determined by the potentiometric titration method. The potentiometric titrator GT-05 (Mitsubishi Kagaku Co., Tokyo) was used. A 100ml solution of STFX in 2mol/l HCl was titrated with 0.1mol/l KOH at 25°C. The pKa values were calculated with the computer program, PKAS.5

Detection of Free Radical and Active Oxygen during Photodegradation by ESR Spin-TRapping

ESR measurements were recorded on a JEOL JES-PE2XG spectrometer (JEOL) with 100kHz field modulation operating at 9.42GHz and at room temperature. The following instrumental parameters were employed: modulation amplitude, 0.063mT; microwave power, 8.0mW; scan time, 2 min.

STFX solution (2.0mg/ml pH 4, 50mm citrate buffer solution) containing DMPO (ca. 1.80m; 200µl) in the presence or absence of 0.2M NaCl was irradiated with D65 fluorescent lamps at approximately 4000lux for 20min. The resultant solution was transferred to capillary tubes (Drummond MI-CROCAPS: 50µl), sealed with TERUMOSEAL (Terumo, Tokyo), and measured by ESR.

Results and Discussion

Structural Elucidation of Photodegradation Products of STFX

STFX was decomposed rapidly in aqueous solution by photoirradiation. Figure 2 shows the HPLC chromatogram of STFX aqueous solution after being photoirradiated by fluorescent lamps for 350000lux·h. Two major photodegradation products eluted around 22 and 24 min were observed in the chromatogram and designated P-1 and P-2, respectively. These products were isolated and identified by 1H-NMR and MS spectra. The product P-1 was identified as 7-[7-aminoo-5-azaspiro[2,4]heptan-5-yl]-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid from the spectrum analyses: field desorption (FD)-MS and FAB-MS m/z: 318 (M+H)⁺, 1H-NMR (CD3COOD): 0.92, 1.19 (2H, m), 1.05 (2H, m), 3.37 (1H, d), 3.67 (1H, d), 4.12 (2H, m), 4.24 (1H, m), 6.80 (1H, d), 7.88 (1H, d), 8.74 (1H, s). The results showed that a chlorine at the 8-position and a cyclopropyl group at 1-position were eliminated. The product P-2 was identified as 1-(1-amino-2-[[6-fluoro-1-(2-fluoro-1-cyclopropyl)-1,4-dihydro-4-oxo-3-quinolin-7-yl]-amino]ethyl)cyclopropenecarbaldehyde: CI-MS m/z: 391 (M+H)+, FAB-MS m/z: 391 (M+H)+, 1H-NMR (D2O+TFPA): 0.14—0.33 (4H, m), 0.53, 0.58 (1H, m), 0.70 (1H, m), 2.14 (1H, m), 2.67 (1H, m), 2.77 (2H, m), 4.01, 4.14 (1H, m), 6.07 (1H, s), 7.24 (1H, s), 7.84 (1H, s). The P-2 product had the structure of STFX oxidized at the 7-position and with a chlorine eliminated at the 8-position. These structures of P-1 and P-2 are shown in Fig. 3. Both compounds contained a dissociated C=Cl bond at the 8-position.

Effect of pH on the Photodegradation

In the case of ionizable compounds, the effect of pH on the photostability is significant. Figure 4 shows the first-order plots for the photodegradation reactions conducted at several pHs. The photodegradation of STFX followed an apparent first-order rate equation in the pH range of 2.0 to 12.9. The first-order plots exhibited good linear relationships with correlation coefficients of 0.99 or higher at all pHs examined. The apparent first-order rate constants (k) were obtained from the slopes of the plots (Table 1). The logk–pH profile for the photodegradation of STFX is shown in Fig. 5. The photodegradation rates in neutral solution were higher than those observed in acidic and alkaline solutions. STFX is an amphoteric compound with pKa values of 5.7 for the carboxylic group and 9.0 for the amine determined by potentiometric titration. This acid dissociation is shown in Chart 1. The isoelectric point of STFX is pH 7.0.
the zwitter ion at pH 7.4. STFX was the most sensitive to photodegradation in zwitter ionic form at slightly basic pHs. STFX was stable in the acidic region, where the carboxyl group was not ionized and the basic nitrogen was completely protonated.

**Wavelength Dependence of Photodegradation** Generally, so-called photo-labile compounds decompose under light of specific wavelengths and thereby contribute to photodegradation of the compounds. Figure 6a shows the photodegraded ratio of STFX after exposure to light of various wavelengths at the same energy (0.5 J/cm²); it clearly shows that STFX was degraded wavelength dependently. The degradation rate and UV spectrum of STFX (Fig. 6b) correlated well. That is, STFX was the most labile at 284 nm, which was the maximum absorption wavelength.

**Mechanistic Consideration of Photodegradation of STFX** In some fluoroquinolones the structures of the photodegradation products have been already identified, such as levofloxacin (LVFX) and orbifloxacin (ORFX). These products are analogues altered at the piperazine moiety or having the fluorine eliminated at the 8-position of fluoroquinolones. STFX was photodegraded in aqueous solution and gave two major products. The structures of the photodegradation products had in common the chlorine eliminated at the 8-position of STFX and in being subsequently degraded at the 1-position or 7-position of STFX. In general, mechanisms by which photodegradation of the compounds
may occur are classified as follows. The Type I mechanism is a free radical chain process and is generally termed autoxida-
tion. The Type II mechanism involves the excited $^1O_2$ and is termed oxygenation. It is well known that heterocyclic com-
ounds such as pyrrole and furan undergo photooxidation by a Type II mechanism. We demonstrated that STFX and the
other fluoroquinolones generated $^1O_2$ in response to photo-
irradiation. We concluded from these that $^1O_2$ production by
irradiated STFX was responsible for photodegradation. STFX reacts with $^1O_2$ to form a dioxetan intermediate, and then undergoes hydrolysis to afford the photodegradation products P-1 and P-2, as presented in Chart 2 according to the Type II mechanism. However, details of the mechanism of the photochemical reaction of STFX are not as clear.

**Effect of Halide Ion on the Photodegradation** ORFX, a fluoroquinolone having a fluorine at the 8-position, was
converted into a photoprodut substituted by chlorine at the 8-position in an aqueous solution containing chlorine ions. We concluded from these that $^1O_2$ production by irradiated STFX was responsible for photodegradation. STFX reacts with $^1O_2$ to form a dioxetan intermediate, and then undergoes hydrolysis to afford the photodegradation products P-1 and P-2, as presented in Chart 2 according to the Type II mechanism. However, details of the mechanism of the photochemical reaction of STFX are not as clear.

### Table 2. Apparent First-Order Rate Constants ($k$) for the Photodegradation of STFX in pH 4.0 Citrate Buffer in the Presence of Chloride Ion, Bromide Ion, and in the Absence of Halide Ion (Control)

<table>
<thead>
<tr>
<th>Condition</th>
<th>$k$ (h$^{-1}$)</th>
<th>Ratio to Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without halide ion</td>
<td>0.733</td>
<td>1.00</td>
</tr>
<tr>
<td>With 0.2M NaCl</td>
<td>0.040</td>
<td>18.4</td>
</tr>
<tr>
<td>With 0.2M NaBr</td>
<td>0.739</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*Ratio to control = 1.00.

or higher. The apparent first-order rate constants ($k$) were obtained from the slopes of the plots. Table 2 shows the $k$ for the photodegradation of STFX in the pH 4.0 citrate buffer solutions containing chloride ion, bromide ion or without halogen ion (control). The photostability of STFX was improved approximately 18 fold in the presence of chloride ion, while it was not affected in the presence of bromide ion.

**LC-MS Analysis of Photodegradation Product in the Presence of Bromide Ion** Figure 8 shows a high-performance liquid chromatogram of STFX photoirradiated in pH 4.0 citrate buffer containing bromide ion with a D$_65$ fluorescent lamp for 0.5 h. Quasi-molecular ions at $m/z=454$ and $m/z=456$ were present in the LC-ESI mass spectrum. Therefore, the structure of the newly observed photodegradation product of STFX in the presence of bromide is the 8-bromo form of STFX, which is substituted for chlorine as shown in Chart 3.

**Kinetics of the Photodegradation in the Presence of Chloride Ion at Several pHs** Figure 9 shows the first-order plots for the photodegradation of STFX in 0.1 M
HClO₄, pH 7.0 phosphate buffer, and 0.1 M NaOH in the presence and absence of chloride ion. Table 3 and Fig. 10 show $k$ for the photodegradation of STFX in the presence and absence of chloride ion in the above solutions. The photostability of STFX was improved approximately 31 fold in the presence of chloride ion in 0.1M HClO₄. Conversely, it was not affected in the presence of chloride ion in 0.1 M NaOH. Furthermore, the photodegradation rate in a neutral solution was almost equal to that in an alkaline solution in the presence of chloride ion.

Fluoroquinolones generated ·C and ·OH in the process of photodegradation in pH 7.0 buffer by means of the 5,5-dimethylpyrroline-1-oxide (DMPO) spin trapping method. The 8-fluorine-substituted fluoroquinolones such as lomefloxacin and sparfloxacin produced ·C; however, STFX did not produce the ·C in pH 7.0 buffer. Figure 11a shows the ESR spectrum of photoirradiated STFX in pH 4.0, and Fig. 11b shows the ESR spectrum of photoirradiated STFX in pH 4.0 in the presence of 0.2 M NaCl. Figure 11a shows that STFX generated ·C and ·OH in the process of photodegradation in pH 4.0 buffer. Therefore, the homolytic bond scission of the C–Cl took place in the absence of chloride ion in pH 4.0 buffer solution. On the contrary, STFX did not generate the ·C in the presence of 0.2 M NaCl in pH 4.0 buffer (Fig. 11b). These results suggest that the chloride ion competes with DMPO in reacting with the generated ·C.

The Mechanism of the Increments in Photostability in the Presence of Chloride Ion

The photostability of STFX
increased in the presence of chloride ions. In their absence, · C was generated at pH 4.0 by homolytic bond scission of the C–Cl bond; however, · C was not observed in the presence of chloride ions. These results might arise because the dechlorination reaction, the initial process of photodegradation, was suppressed in the presence of chloride ions. The · C was generated and degraded into P-1 and P-2 by photoirradiation in the absence of chloride ions; but, in the presence of these ions, · C immediately reacted with them. As a result, the C–Cl bond was recovered, and the apparent photostability was increased. Furthermore, the photodegradation rate of STFX was not affected by the presence of bromide ions. A new major photodegradation product was the 8-bromo form of STFX, which was not observed in the absence of bromide ions, indicating that the dissociation of the C–Cl bond at the 8-position of STFX is the rate-limiting step in the initial process of photodegradation of STFX. Postulated mechanisms for the photodegradation of STFX solution in the presence of chloride ions and bromide ions are summarized in Chart 4.

In conclusion, the photodegradation behavior of STFX was influenced by the addition of chloride ions. The photostability of STFX was increased in the presence of chloride ions in acidic and neutral solutions.

References