Histamine-Releasing Properties of T-3762, a Novel Fluoroquinolone Antimicrobial Agent in Intravenous Use. II.1) Dermovascular Permeability-Increasing Effect and Action on Peritoneal Mast Cells

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To predict the actions of T-3762, a newly developed fluoroquinolone antimicrobial agent, as well as ciprofloxacin (CPFX) and ofloxacine (OFLX), on injection sites when dosed parenterally, their ability to increase cutaneous vascular permeability in dogs and to release histamine from rat peritoneal mast cells was examined. CPFX and OFLX increased cutaneous vascular permeability in concentrations ranging from 16 to 32 mg/mL, while T-3762 was inactive at 2000 mg/mL. The vascular permeability-increasing activities of these drugs were inhibited efficiently by pretreatment with a combined dose of diphenhydramine and cimetidine. CPFX induced histamine release from rat mast cells in a dose-dependent manner, whereas T-3762 was ineffective. Therefore, it is concluded that fluoroquinolone antimicrobial agents may have the ability to cause an increase in cutaneous vascular permeability by releasing histamine from mast cells at the injection site when administered parenterally, and that T-3762 has minimum activity among the agents tested in this study.

Key words fluoroquinolone; histamine; mast cell; cutaneous vascular permeability; dog; rat

Parenteral fluoroquinolone antimicrobial agents, if available, are promising for the empiric therapy as well as definitive therapy of infectious diseases because they have a broad spectrum of antimicrobial activity with relatively few serious side effects. Nevertheless, it has been reported that the intravenous administration of ciprofloxacin (CPFX) frequently causes erythema, itching and a burning sensation at the injection site.2-5) Furthermore, fluoroquinolones, including CPFX,6) ofloxacine (OFLX)7) and levofloxacine (LVFX),8) have the ability to rapidly reduce blood pressure after intravenous administration to dogs. Previous studies have suggested that histamine release may involve local and systemic responses to parenterally administered fluoroquinolones.9-13) In the preceding work,9,10) we confirmed that hypotension and an increase in plasma histamine concentration occurred dose-dependently with a good relationship between them when either CPFX and OFLX was administered intravenously to anesthetized dogs. In addition, T-3762 (Fig. 1), a newly developed antimicrobial fluoroquinolone for parenteral use,9,10) was virtually free of cardiovascular activity or the ability to increase concentrations of histamine in plasma, despite its close similarity in chemical structure to CPFX and OFLX.

In the present study, therefore, we tested the hypothesis that parenteral fluoroquinolones would cause a cutaneous vascular permeability increase at the site of injection through their direct action to mast cells from which histamine, in turn, is released.

MATERIALS AND METHODS

Animals Male and female mongrel adult dogs weighing 12 to 20 kg were purchased from K. K. Chubu (Nagoya, Japan) and male Wistar/ST rats weighing 270 to 340 g were from Japan SLC (Hamamatsu, Japan). Animals were acclimatized in the breeding room under the following conditions: temperature, 22±2°C; humidity, 60±10%; light-on and light-off cycle, 12 h (light-on period: 6:00 to 18:00). They were given commercially available solid food and water.

Drugs T-3762 was synthesized in this laboratory by the method described previously.9,10) CPFX was obtained by extracting it from the Ciprofax tablets of Bayer Co. (Osaka, Japan), and was used after purification. OFLX was the commercial product of Sigma Co. (St. Louis, U.S.A.). For injection, these agents were dissolved in HCl and physiological saline (Otsuka, Tokyo, Japan) to adjust the concentrations to 2000 mg/mL (osmotic pressure ratio: 1, pH 3.5—3.6), then serially diluted with saline. In in vitro experiments, they were dissolved in HCl and physiological salt solution-B (PSS-B) containing 145 mm NaCl, 2.7 mM KCl, 5.6 mm glucose, 1.0 mm CaCl2, and 20 mm HEPES (pH 6.5, Nacalai Tesque, Kyoto, Japan), and the pH was adjusted to 6.5 with NaOH. Diphendydramine hydrochloride (DPH, Sigma) and cimetidine (CMT, Fujisawa, Osaka, Japan) were dissolved with saline to concentrations of 3 and 10 mg/mL, respectively. PSS-A, containing 5.0 mm HEPES instead of 20 mm, in PSS-B, at pH 7.4, was used as a buffer solution. The following reagents, all of analytical grade, were used: acetonitrile, n-heptane, chloroform (for HPLC, Wako, Osaka, Japan), o-phthalaldehyde (OPA), bis(2-ethylhexyl)hydrogenphosphosphate, sodium 1-heptanesulfonate, glutaraldehyde solution for electron microscopy, osmic acid solution for electron microscopy, isoamyl acetate (Wako), compound 48/80 (Sigma), Percoll (Pharmacia, Tokyo, Japan) and di-sodium hydrogen citrate (Nacalai Tesque).

Methods for Cutaneous Vascular Permeability Cutaneous vascular permeability was measured according to the
methods of Edward and Katayama with a slight modification. Dogs were anesthetized with sodium pentobarbital. The lateral region was shaved using hair clippers. After 2 h, 0.1 ml of each solution for the test drugs from 4 to 2000 μg/ml, was administered intradermally in the shaved skin area at arbitrary intervals, then 2% Evans blue solution was intravenously injected at 1 ml/kg. Thirty minutes after the injection, the lateral region of the animals was photographed, and the parts of the skin including the injection sites were punched. The skin samples thus collected were cut into thin sections and left in 1 ml of 1 N-KOH at 50°C for 18 h. After 9 ml of 0.6 N-H₃PO₄ acetone solution (5:13) was added, the sections were centrifuged at 1800 × g for 15 min. The dye concentrations in the supernatants were quantified at 620 nm by a spectrophotometer (UV-160, Shimadzu). In one experiment, CMT (10 mg/kg) and DPH (3 mg/kg) were simultaneously administered intravenously 15 min before the intradermal administrations.

**Methods for Histamine Release from Mast Cells**

Rats were exsanguinated by decapitation, then 15 ml of PSS-A solution was intraperitoneally injected. After the abdomen was massaged for 90 s, the peritoneal fluid was collected. The fluids collected from 6 rats were combined and centrifuged at 5500 × g for 8 min at 4°C. The sedimented cells were suspended in 1 ml of PSS-A and treated with 3 ml of 88% Percoll. After a further addition of 1 ml of PSS-A to the suspension, followed by centrifugation at 2650 × g for 15 min at 4°C, mast cells were suspended in PSS-B at 1 × 10⁶ cells/ml. Mast cell counting was performed by the routine method using toluidine blue staining. To determine the amount of histamine released, 630 μl of the test solution in each concentration was added to 70 μl of the cell suspension, and the mixture was incubated at 37°C for 5 min. The reaction was stopped by the addition of 700 μl of ice-cooled PSS-B to the mixture, and the supernatant was immediately separated by centrifugation at 3000 × g for 10 min at 4°C. The survival rate of the cells was determined by the trypan blue dye exclusion method. The sedimented cells were suspended in 1.2 ml of the buffer and disrupted by boiling for 5 min, then the supernatant was obtained by centrifugation at 7800 × g for 10 min at 4°C. The histamine concentrations in the supernatants of both the incubation mixture and the ruptured cells were quantified by HPLC.

**Histamine Quantification**

To 0.5 ml of the supernatant sample, 0.5 ml of 0.05 M phosphate buffer, pH 7.4, and 4 ml of chloroform were added, and the mixture was then shaken for 2 min in the cold and centrifuged to separate the aqueous layer, from which contaminants were removed by successive extraction with 0.05 M bis(2-ethylhexyl)hydrogenphosphate/n-heptane at neutral and acidic pH before HPLC. To 0.2 ml of this aqueous layer, 40 μl of 1 N-NaOH and 10 μl of 0.1% OPA in methanol were added, left at 10°C for 10 min, then mixed with 20 μl of 3 N-HCl before HPLC.

The HPLC attached to the following Shimadzu apparatus was used: LC-10 type pump, RF-10A type spectrophotometer (Ex 355 nm, Em 450 nm) and STR ODS-II column (4.6 × 200 mm, 30°C). The shift phase consisted of 10 mm sodium citrate/18% acetonitrile/0.1% sodium 1-heptanesulfonate, and the flow rate was 1.0 ml/min.

**Mast-Cell Preparations for Morphological Observation**

The cells obtained after incubation were fixed in 2% glutaraldehyde, postfixed in 1% osmic acid, and then successively dehydrated with 50% to 100% ethanol and 100% isoamyl acetate. After drying at a critical point, gold was evaporated and the cells were subjected to examination by scanning electron microscopy (SEM, Hitachi S4500).

**Statistical Analysis**

The results are expressed as the mean±standard error. Significance was tested using Dunnett’s multiple comparison or Student’s t-test, with p<0.05 regarded as significant.

**RESULTS**

**Effects on Cutaneous Vascular Permeability in Dogs**

As shown in Fig. 2A, CPFX and OFLX caused graded dye-extravasation by the intradermal injections in concentrations from 16 to 32 μg/ml or more, while T-3762 was only effective in a high concentration of 2000 μg/ml in 2 of 4 dogs. In accordance with this photo, either CPFX, and to a lesser extent OFLX, increased dye-leakage quantitatively as the concentrations increased, whereas T-3762 at all concentrations employed did not elevate the leaked amounts of dye above the vehicle level (Fig. 3A). Pretreatment of the animals with antihistamines produced a marked inhibition of the CPFX- and OFLX-induced extravasation of dye (as noted in Fig. 2B and depicted quantitatively in Fig. 3B).

**Actions on Rat Peritoneal Mast Cells**

Activities which induced histamine release from rat peritoneal mast cells were

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Fig. 2. Dye-Extravasation in the Lateral Skin Region after Intradermal Injection of Fluoroquinolones Followed by Intravenous Administration of 2% Evans Blue in Dogs

A: Administration of a fluoroquinolone alone. B: Intravenous treatment with diphenhydramine hydrochloride (3 mg/kg) and cimetidine (10 mg/kg) in combination 15 min before the fluoroquinolone injection.
compared between T-3762, CPFX and compound 48/80, a well-known secretogogue, and the result is summarized in Fig. 4. CPFX exhibited a significant capacity for releasing histamine from the cells at 400 and 800 μg/ml, while T-3762 was ineffective at the maximum concentration of 800 μg/ml. Compound 48/80 caused a liberation of the intracellular histamine by 73.5% at 3 μg/ml in the incubation medium. As for the survival rate of the cells after 5 min incubation, CPFX and T-3762, in concentrations ranging from 100 to 800 μg/ml, were not significantly different from the vehicle (Table 1). However, the cells incubated with CPFX at 400 μg/ml demonstrated some morphologic alterations, such as bleb-like protrusions and the disappearance of microvilli; to a varying degree, in the surface of the cell membrane, while those with T-3762 at the same concentration remained intact in appearance as compared to the controls with the vehicle (Fig. 5).

DISCUSSION

Cutaneous vascular permeability was increased gradually by the intradermal injection of either CPFX or OFLX at more than 16 μg/ml. The increases were totally prevented in animals pre-treated with antihistamines. The in vitro experiments using rat peritoneal mast cells revealed that CPFX at more than 400 μg/ml was capable of releasing histamine from the cells, in association with certain morphological alterations in the cell membrane, without resultant cell death. Nevertheless, the concentration required for the induction of

![Fig. 3. Effects of Fluoroquinolones on Cutaneous Vascular Permeability in Dogs](image)

The fluorquinolones were intradermally administered just before the 2% Evans blue solution was intravenously injected. The dye was extracted from skin samples taken 30 min later, and extinction was measured photometrically at 620 nm. Each point represents the mean±S.E. of 4 animals. A: Administration of a fluorquinolone alone. B: Intravenous treatment with diphenhydramine hydrochloride (3 mg/kg) and cimetidine (10 mg/kg) in combination, 15 min before the fluorquinolone-injection. Statistical significance was shown as follows: significantly different from the T-3762, * (p<0.05) and ** (p<0.01); and from the vehicle, * (p<0.05) and ** (p<0.01), by Dunnett's multiple comparison test.

![Fig. 4. Effects of T-3762 and CPFX on Histamine Release from Rat Peritoneal Mast Cells](image)

Each column represents the mean±S.E. (n=4). **: p<0.01, significant differences from the vehicle by Dunnett's multiple comparison test.

Table 1. Survival Rate of Rat Peritoneal Mast Cells after a 5 min Incubation with Fluoroquinolones

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Concentrations (μg/ml)</th>
<th>Viability (%)±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100</td>
<td>89.9±2.0</td>
</tr>
<tr>
<td>T-3762</td>
<td>200</td>
<td>89.1±6.7</td>
</tr>
<tr>
<td>CPFX</td>
<td>400</td>
<td>84.2±2.7</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>80.9±5.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>82.2±4.6</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>86.2±4.9</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>88.5±1.7</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>85.7±3.7</td>
</tr>
</tbody>
</table>

a) Unstained cells treated with 0.075% trypan blue for 3 min were regarded as viable cells. Each value represents the mean±S.E. (n=4).

![Fig. 5. Morphological Changes in Rat Peritoneal Mast Cells after a 5 min Incubation with Medium Alone (A), T-3762 at 400 μg/ml (B), or CPFX at 400 μg/ml (C)](image)
histamine release from mast cells is higher than that for the cutaneous permeability increase in vivo. This may be due to a species difference in the responsiveness of mast cells to fluoroquinolones.\textsuperscript{13} Besides, the concentrations of CPFX and/or OFLX cutaneously in this study are fairly comparable to those described in the preceding study,\textsuperscript{1,14} in terms of producing a rapid increase in plasma histamine concentration accompanying hypotension after infusion into the veins of dogs. Therefore, it appears likely that intravenously injected quinolones may diffuse easily into the extravascular space to trigger the release of histamine from tissue mast cells, which in turn causes vasodilation and a vascular permeability increase in the microvasculature, leading to hypotension. This assumption does not exclude the possibility of the direct participation of basophils in increasing plasma histamine concentrations when quinolones are intravenously administered. In the present study, T-3762 is shown to be practically free of skin irritation and histamine releasing activities, when compared with CPFX and OFLX. This does not conflict with the results of our preceding study,\textsuperscript{11} which showed that the compound had no effect on blood pressure, heart rate or plasma histamine concentration following intravenous infusion in anesthetized dogs. Although the basic ring structure of the 4-quinolone is the same, the T-3762 molecule differs from both CPFX and OFLX regarding the kinds of substituents and their positions of substitution; in particular, it is devoid of the piperazinyl moiety in the 7 position, which many already known fluoroquinolones, including CPFX and OFLX, possess. Since T-3762 contains a 1-aminocyclopropyl moiety at position 7, the differences in the present and preceding studies may be largely contribute to the reduction of pharmacological activities characteristically belonging to CPFX and OFLX. This assumption should be tested in more definitive experiments concerning this structure activity relationship.

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\textbf{REFERENCES AND NOTES}