Histamine-Releasing Properties of T-3762, a Novel Fluoroquinolone Antimicrobial Agent in Intravenous Use. I. Effects of Doses and Infusion Rate on Blood Pressure, Heart Rate and Plasma Histamine Concentration

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T-3762, a newly developed fluoroquinolone antimicrobial agent, ciprofloxacin (CPFX) and ofloxacin (OFLX) were administered intravenously to anesthetized dogs by intravenous infusion, and blood pressure, heart rate and plasma histamine concentrations were monitored. T-3762 decreased blood pressure by 12.0%, without alterations in heart rate and plasma histamine concentration, only when infused at 150 mg/min. CPFX and OFLX both produced a rapid decrease in blood pressure in a dose-related manner, with an accompanying decrease in heart rate, but to a lesser extent. After infusion at 150 mg/min, CPFX caused death in 2 animals within a few minutes, while OFLX produced maximum decreases in blood pressure and heart rate, by 69.0% and 26.4%, respectively. The infusion of these 2 agents resulted in dose-related increases in plasma histamine concentrations parallel to the decreases in blood pressure: the maximum, attained with CPFX at 50 mg/min and OFLX at 150 mg/min, were 379.2 and 167.8 ng/mL, respectively. For CPFX and OFLX, the relationship between the maximum levels of decreased blood pressure and increased histamine concentration in plasma was highly significant. The hypotension induced by CPFX was efficiently reduced by the pretreatment of animals with antihistamines. The results from this study suggest that hypotension induced in dogs following the intravenous infusion of fluoroquinolone antimicrobial agents may be dependent on their ability to cause histamine release from cells and tissues, and indicates that T-3762 is devoid of this ability in comparison to CPFX and OFLX.

Key words fluoroquinolone; hypotension; histamine; dog

In practical use for the treatment of systemic infectious diseases in hospitalized patients, antimicrobial agents are necessary to rapidly attain maximum therapeutic concentrations in plasma without any adverse side effects. Fluorinated quinolone compounds, with a broad spectrum of antimicrobial activity, are used by oral administration for the treatment of a wide variety of infectious diseases caused by susceptible microorganisms, including urogenital infections, respiratory tract infections, bone-joint-soft tissue infections and others. If rapidly administered by the parenteral route, however, fluoroquinolones, such as ofloxacin (OFLX), levofloxacin (LVFX) and norfloxacin (NFLX), have been reported to cause an immediate decrease in blood pressure in anesthetized dogs.1−3) or to produce erythema, itching and a burning sensation at the injection sites in the arms of healthy volunteers who received ciprofloxacin (CPFX).4,5) Previous reports suggest the involvement of systemic and local histamine release in the hypotension and skin-irritation induced by the parenterally administered quinolones.1−3,6) Pauzoxacin is a novel fluoroquinolone for oral use, which has been estimated as efficacious in antimicrobial activity as OFLX and CPFX in vivo and in vitro, as well as less toxic with special reference to acute toxicity, mammalian cell cytotoxicity, inhibition of calf thymus topoisomerase II and the induction of convulsions in mice.7−9) T-3762 (Fig. 1) is the methanesulfonate of pauzoxacin,10) and is more easily soluble in water. The present study was designed to clarify whether parenteral fluoroquinolone-induced hypotension in anesthetized dogs might be related to the increase in plasma histamine concentrations and/or to the rate of doses for injection, and, in this respect, whether any differences exist between T-3762, OFLX and CPFX.

MATERIALS AND METHODS

Animals Male and female mongrel adult dogs (body weight: 9 to 19 kg, purchased from K. K. Chubu, Nagoya, Japan) were used. The animals were acclimatized in the breeding room under the following conditions: temperature, 22±2°C; humidity, 60±10%; lighting, on and off at 12-h intervals (lights-on period: 6:00—18:00). Commercially available solid food and water were given ad libitum.

Drugs T-3762 was synthesized in this laboratory by the method described previously.8,10) CPFX and OFLX were both extracted from commercial products and used after purification and identification. T-3762 was dissolved to a concentration of 30 mg/ml in distilled water, and in saline solution (pH 2.7, osmotic pressure ratio 1.1). CPFX and OFLX were dissolved in hydrochloric acid and saline solution to a concentration of 30 mg/ml (CPFX: pH 2.4, osmotic pressure ratio 1.1, OFLX: pH 5.6, osmotic pressure ratio 1.1). The solutions were further diluted with saline, and those containing the test compounds at 10 mg/ml were prepared. Diphenhydramine hydrochloride (DPH, Sigma, St. Louis, U.S.A.) and cimetidine (CMT, Tagamet, Fujisawa, Osaka, Japan) were dissolved in saline at 3 and 10 mg/ml, respectively. Acetoni-trile, methanol, chloroform, and n-heptane were of HPLC quality, and o-phenylaldehyde (OPA), bis(2-ethylhexyl)hydro-

Fig. 1. Chemical Structure of T-3762

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genephosphate and sodium 1-heptanesulfonate were purchased from Wako Pure Chemical Industries (Osaka, Japan).

**Measurements of Blood Pressure and Heart Rate, and Collection of Blood Samples** Animals were anesthetized by an intravenous injection of sodium pentobarbital and tracheotomized for airway cannulation. The blood pressure of the left femoral artery was measured with a pressure transducer (P-23, Statham) and heart rates were calculated from blood pressure signals. These data were simultaneously recorded with a polygraph (Polygraph 360 system, Sanei Sokuki). The basal levels of blood pressure and heart rate determined from a total of 46 dogs prior to administration were 141.9 ± 2.4 mmHg and 176.6 ± 3.9 beats/min, respectively. The values obtained in each experiment were expressed as those relative to the basal levels (0%). A single dose of each drug, 1 ml/kg, was continuously infused through the cannula inserted into the right femoral vein either at a slow rate of 0.33 ml/min for approximately 45 min or at a rapid rate of 5 ml/min for 5 min. Blood samples, 10 ml each, were collected from the catheter retained in the femoral vein for measurement of histamines. In pretreatment with antihistamines, the animals received a combination of 3 mg/kg of DPH and 10 mg/kg of CMT intravenously 15 min prior to the start of the infusion.

**Plasma Histamine Determination** Plasma histamine was measured by the method of Tsikas et al. with a slight modification. Briefly, plasma (0.5 ml) was mixed with 0.5 ml of 0.05 M phosphate buffer (pH 7.4) and 4 ml of chloroform. The mixture was centrifuged at 2000 × g for 5 min at 5°C after shaking for 2 min. The supernatant (0.9 ml) was shaken with 5 ml of 0.05 M bis(2-ethylhexyl) hydrogen phosphate/n-heptane for 10 min. After centrifugation at 2000 × g for 15 min at 5°C, the supernatant was acidified with 0.45 ml of 0.1 N HCl and shaken for 10 min. The aqueous layer, 0.2 ml, was pipetted out and maintained at 10°C for 10 min in the presence of 1 N NaOH (40 μl) and 0.1% OPA/methanol (10 μl) and then subjected to HPLC-analysis after the addition of 3 N HCl (20 μl). The HPLC system consisted of an L-6000 type pump (Hitachi), an RF-10A spectroscopy fluorescence detection device (Ex 360 nm, Em 450 nm, Shimadzu) and an STR ODS-II (200×4.0 mm, 3 μm, Shimadzu) column. The mobile phase was acetonitrile, 1 M citric acid 2 sodium–NaOH (pH 5.0), distilled water, and 1-heptanesulfonic acid sodium (180 ml : 10 ml : 810 ml : 1 g), and the flow rate was maintained at 1.0 ml/min.

**Statistics** Statistical evaluation of the data was performed using Student's t-test or Dunnnett's multiple comparison test. A value of p < 0.05 was considered significant.

**RESULTS**

**Effects of Slow-Rate Infusion (Fig. 2)** At either 10 or 30 mg/kg, T-3762 did not cause any changes in mean blood pressure, heart rate or plasma histamine concentrations. However, CPFX produced a decrease in blood pressure immediately after the start of the infusion, which lasted for at least 60 min; the maximum decreases were 22.6% at 10 mg/kg and 36.5% at 30 mg/kg. Heart rates also tended to decrease in all animals given CPFX, with the exception of a slight transient increase which occurred very early in 3 of 4 dogs given the 30 mg/kg dosage. Plasma histamine concentrations were increased by infusion at both 10 and 30 mg/kg, and reached averages of 10.2 and 71.9 ng/ml, respectively, until 10 min, then decreased gradually to the initial levels for up to 60 min. Similarly, OFLX produced a rapid and sustained decrease in blood pressure at a maximum of 30.5% at the high dose of 30 mg/kg, although the effect was not clear at 10 mg/kg. The heart rate was not affected by OFLX at 10 mg/kg, but showed a slight decrease from the initial rate during 30 mg/kg infusion. At the high dose, plasma histamine concentrations increased to a plateau of about 6.9 ng/ml, which was maintained from 10 min until the cessation of infusion, while the low dose had no effect.

![Graphs showing effects of different drugs on blood pressure and histamine concentration](image)

**Fig. 2. Effects of the Quinolones on Mean Blood Pressure (MBP), Heart Rate (HR), and Plasma Histamine Concentration in Anesthetized Dogs Following Intravenous Infusion at a Slow Rate**

Each point represents the mean ± S.E. of 4 animals. **p<0.01, *p<0.05**, significant difference from T-3762 by non-parametric Dunnnett's multiple comparison test (2-side).
Fig. 3. Effects of the Quinolones on Mean Blood Pressure (MBP), Heart Rate (HR), and Plasma Histamine Concentration in Anesthetized Dogs Following Intravenous Infusion at a Rapid Rate.

Each point represents the mean±S.E. of 4 animals. The data for CPFX at 30 mg/kg were derived from 2 live animals. ++: p<0.01, *: p<0.05, significant difference from T-3762 by non-parametric Dunnett's multiple comparison test (2-side).

Fig. 4. Effects of Pretreatment with CMT and DPH on Hypotension and Plasma Histamine Concentration Induced by the Rapid Infusion of T-3762 and CPFX in Anesthetized Dogs

CMT (10 mg/kg) and DPH (3 mg/kg) were simultaneously injected intravenously 15 min before the start of the infusion. Each column represents the mean±S.E. of 4 animals. *: p<0.05, significantly different from the untreated; Student's t-test was used.

Effects of Rapid-Rate Infusion (Fig. 3) Immediately after the start of the infusion of CPFX, blood pressure as well as heart rate decreased markedly, and 2 animals given CPFX at 30 mg/kg died within a few minutes. Plasma histamine concentrations increased sharply to 379.2 ng/ml in animals given 10 mg/kg and to 1375 ng/ml in those given 30 mg/kg. The blood pressure and heart rate in the low-dose group gradually returned to normal, but each still remained below the initial level at the termination of the 120-min observation period. OFLX also produced a marked decrease in blood pressure immediately after the start of the infusion, followed by a gradual recovery to the initial level; the maximum hypotensive responses were 41.5% at 10 mg/kg and 69.0% at 30 mg/kg. Heart rates were decreased maximally by 26.4% at 30 mg/kg, but the effect at 10 mg/kg was almost negligible. However, OFLX at the low dose caused a rapid increase in plasma histamine concentration, to a maximum level comparable to that induced by the high dose (140 vs. 168 ng/ml), with a relatively rapid return to the initial level. T-3762 transiently decreased blood pressure by 12.0% very early after the start of infusion at 30 mg/kg. There were no other specific alterations at 10 mg/kg.

Effects of Pretreatment with Antihistamine As shown in Fig. 4, pretreatment with a combined dose of CMT and DPH efficiently prevented the development of hypotension following the rapid infusion of CPFX at 10 mg/kg. However, the pretreatment was ineffective against the increase in plasma histamine concentration due to CPFX. Pretreatment
did not influence blood pressure or histamine concentrations in plasma after a rapid infusion of T-3762 at 30 mg/kg.

The Relationship between the Administration Rate, Hypotension, and the Plasma Histamine Concentration

As shown in Fig. 5, the maximal hypotension rate caused by CPFX, OFLX and T-3762, respectively, depended on the administration rate. Figure 6 shows that either CPFX or OFLX, but not T-3762, produced an exact correlation between blood pressure and histamine concentration in plasma.

DISCUSSION

The present study demonstrated that either CPFX or OFLX decreased blood pressure, and to a lesser extent heart rate, in association with a parallel increase in plasma histamine concentration after the start of the infusion at a slow or rapid rate through a cannulated vein in anesthetized dogs. With these quinolones, the maximum decrease in blood pressure and increase in histamine concentration in plasma appeared together within at least 10 min after infusion, and were apparently proportional to the speed of infusion as well as the concentrations of quinolones in the infusates. Analyses of the data indicated that the degree of hypotension induced with the quinolones including T-3762 correlated well with the amounts infused in a time-unit of minutes. They also revealed a statistically good relationship between the maximum decrease in blood pressure and maximum increase in plasma histamine concentration for CPFX and OFLX. These quinolones may directly release histamine into the circulation, which in turn causes hypotension in anesthetized dogs. This assumption is supported by the results of experiments in dogs pretreated with antihistamines, and, in addition, from the findings of a preliminary experiment in which tachyphylaxis rapidly developed following the repeated administration of quinolones (data not shown).

Following rapid intravenous administration of a relatively large dose of vancomycin, the clinically well-known red neck syndrome is produced in frequent association with hypotension and myocardial depression. The underlying mechanism of histamine release is thought to be responsible for this phenomenon.12—14 Moreover, the severity of the syndrome has been reported to be correlated with the area under the plasma histamine concentration-time curve (AUC).15 On the other hand, in a recent study, this correlation was not statistically significant.13 As shown in Fig. 3, the peak height of the plasma histamine concentrations attained early during infusion seems more important than the AUC for the prediction of hypotension following the intravenous administration of quinolones in dogs.

In general, commercially available fluoroquinolone antimicrobial agents, when administered parenterally, induce histamine release, especially in dogs, leading to transient hypotension.15 The activities of T-3762, however, were practically negligible compared with those of both CPFX and OFLX under the same experimental conditions. T-3762 lacks the piperazine moiety in the chemical structure which many known fluoroquinolones, including CPFX and OFLX, possess at position 7. Instead, T-3762 contains a 1-aminocyclopropyl moiety in the 7 position of the basic ring structure, which is similar to that of OFLX. Although such chemical modifications may contribute to the reduction of the histamine-releasing and hypotension-inducing activities of both CPFX and OFLX, further studies of the structure-activity relationships between T-3762 and its analogues are needed.

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REFERENCES