J. Pharm. Pharmacol. 2000, 52: 577–584
Received November 1, 1999
Accepted December 21, 1999

Characterization of Histamine Release Induced by Fluoroquinolone Antibacterial Agents In-vivo and In-vitro

KAZUHIKO MORI, CHIKAKO MARU AND KIYOSHI TAKASUNA

Drug Safety Research Laboratory, Daiichi Pharmaceutical Co. Ltd, 16-13 Kitakasai 1-chome, Edogawa-ku, Tokyo 134-8630, Japan

Abstract

Characterization of histamine release induced by fluoroquinolone antibacterial agents, levofloxacin and ciprofloxacin, was investigated in-vivo and in-vitro.

Intravenous injection of levofloxacin and ciprofloxacin at 1–10 mg kg^{-1} produced dose-related elevations in plasma histamine level in anaesthetized dogs. In contrast, levofloxacin was devoid of plasma histamine increment in anaesthetized rats at 100 mg kg^{-1}, whereas ciprofloxacin at the same dose caused endogenous histamine release. Levofloxacin and ciprofloxacin induced non-cytotoxic secretion of histamine from all mast cells tested in a concentration-dependent manner, whereas rat skin and peritoneal mast cells were thirty- to one-hundred-times less sensitive to the effect of fluoroquinolones as compared with the canine skin mast cells.

These results suggest that the functional heterogeneity of mast cells from different species in histamine releasing activity of fluoroquinolones may exist, and that mast cells from the dog appear to be particularly sensitive to the effect of the fluoroquinolones.

Due to the broad antibacterial spectra, bactericidal activity and good pharmacokinetics of the new fluoroquinolone antibacterial agents such as ofloxacin, levofloxacin and ciprofloxacin, these drugs are widely used for the treatment of various infections (Schacht et al 1988; Une et al 1988; Smythe & Rybak 1989). Despite the fact that several fluoroquinolones have been shown to produce severe hypotension when administered intravenously in bolus doses to dogs and cats (Kojima et al 1984; Takasuna et al 1992; Furuhata et al 1998a), there are few reports on adverse cardiovascular reactions to these compounds, such as hypotension, in man (Schacht et al 1988; Smythe & Rybak 1989; Shar & Mulert 1990; Stahlmann 1990). The occurrence of the hypotension caused by fluoroquinolones was reported to be parallel to elevations in plasma histamine concentration (Takasuna et al 1992; Furuhata et al 1998a). Repeated administration of fluoroquinolones to those species gave rise to tachyphylaxis (Kojima et al 1984), and the hypotensive action was blocked by pretreatment with histamine H_1- and H_2-blockers (Furuhata et al 1998a). This suggested that endogenous histamine release due to fluoroquinolones was involved, at least in part, in the hypotensive action. In contrast, large numbers of fluoroquinolones were almost devoid of hypotensive effects in other species such as rats and rabbits, even at doses higher than those administered to dogs (Kojima et al 1984; Takasuna et al 1992).

Histamine is released from tissue mast cells and/or peripheral blood basophils into the circulation by a variety of drugs such as anaesthetics and radiographic contrast media through a direct action on these cells (Genovese et al 1996). Intradermal injections of several fluoroquinolones, including ofloxacin, in dogs have been reported to induce a flush response (Kurata et al 1995) or an increase in cutaneous vascular permeability (Furuhata et al 1998b). Those results indicated that a marked elevation in plasma histamine concentration induced by fluoroquinolones may be mediated by histamine secretion from canine skin mast cells. Similarly, Yoshida et al (1994) have shown that levofloxacin and ciprofloxacin increased permeability of rat skin microvasculature through the induction of histamine release from mast cells. However, the concentrations required for skin reactions in rats was considered to be approximately 15- to 500-times

Correspondence: K. Mori, Drug Safety Research Laboratory, Daiichi Pharmaceutical Co. Ltd, 16-13 Kitakasai 1-chome, Edogawa-ku, Tokyo 134-8630, Japan.

higher than for dogs. Mast cell heterogeneity in their functional response to a variety of stimuli has been found to exist between different species and tissues (Pearce 1982; Lawman et al 1988).

To clarify the histamine releasing properties of fluoroquinolones, we investigated the action of levofloxacin, an optically active isomer of ofloxacin, on histamine release in-vivo and in-vitro, in comparison with that of ciprofloxacin.

Materials and Methods

Chemicals

Levofloxacin and ciprofloxacin were synthesized at Daiichi Pharmaceutical Co., Ltd (Tokyo, Japan). Compound 48/80, collagenase (Type I), hyaluronidase (Type I-S), bovine serum albumin (BSA), 2-deoxy-D-glucose, antimycin A and Triton X-100 were purchased from Sigma Chemical Co. (St Louis, MO). RPMI-1640 medium was obtained from Men-eki Seibutsu Kenkyujo (Gunma, Japan). Heat-inactivated foetal bovine serum (FBS) was a product of Gibco IBL (NY). Percoll was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Tyrode solution (pH 7·4) containing (mM) NaCl 124, KCl 4, NaH_2PO_4 0·64, $NaHCO_3$ 10, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10 and 0·05% BSA. All reagents were of analytical grade.

Animals

Adult beagle dogs of either sex (9–14 kg) and male Sprague-Dawley rats (250–400 g) were purchased from CSK Research Park, Inc. (Nagano, Japan) and Japan SLC, Inc. (Hamamatsu, Japan), respectively. They were housed in air-conditioned rooms (temperature 23 ± 2°C; r.h. 55 ± 20%; 12-h light cycle) and given commercial laboratory chow (DM-2 for dogs, F-2 for rats; Funabashi Farm, Funabashi, Japan), and tap water was freely available. The experimental protocols were in accordance with our institutional guideline for use of laboratory animals.

Effects of fluoroquinolones on plasma histamine concentration in-vivo

The animals were anaesthetized with sodium pentobarbital (Nembutal, Dainippon Pharmaceutical Co., Tokyo, Japan), 30 mg kg^{-1} intravenously for dogs and 50 mg kg^{-1} intraperitoneally for rats. The fluoroquinolones were dissolved in 0·9% physiological saline at concentrations of 0·3–10 mg kg^{-1}, with the pH range 7·1–7·3 for levofloxacin and 4·2–4·5 for ciprofloxacin. The fluoroquinolones were injected into the right saphenous vein for dogs or the right femoral vein for rats over 1 min. The dosage levels were 0·3, 1, 3 and 10 mg kg^{-1} for dogs and 10, 30 and 100 mg kg^{-1} for rats. Control animals received 0·9% saline alone. Compound 48/80, a potent histamine liberator, was used as positive control. Whole blood samples (2 mL) were withdrawn from the left saphenous vein for dogs or the left femoral vein for rats by use of a syringe containing 1% disodium ethylenediaminetetraacetic acid (EDTA-2Na) 5 min after administration of the test compounds, and centrifuged (2000 *g* for 15 min at 4°C). Plasma was collected and stored at −80°C until measurement of histamine. Histamine concentrations in plasma were determined using a commercial ELISA kit (Histamine-ELISA, ICN Pharmaceutical Inc., CA) according to the manufacturers instruction. None of the fluoroquinolones used interfered with the ELISA methods.

Cell preparations

The suspensions of skin mast cell from dog and rat were prepared by the collagenase dispersion method with some modifications, previously described by Pearce & Ennis (1980) and Barret et al (1985). Briefly, the animals were anaesthetized and killed by bleeding. The abdominal skin was shaved and excised, then dissected free of underlying fat, and then chopped with scissors finely into fragments of approximately 1–2 mm^2. The fragments were washed five times with calcium- and magnesium-free Tyrode solution. The fragments were then digested for a predetermined optimum period (180 min for dogs; 150 min for rats) at 37°C with shaking in RPMI-1640 medium (5 mL (g skin)$^{-1}$) containing collagenase (2 mg mL^{-1}) and hyaluronidase (1 mg mL^{-1}) supplemented with 5% FBS. The incubation mixture was gently gassed throughout with 95% O_2 and 5% CO_2. At the end of the incubation, the tissue was disrupted by expression through a syringe, and the resulting suspension was filtered through gauze and washed twice with ice-cold Tyrode solution. Cells were passed through a nylon filter (100-μm pore size, Falcon 2360, Becton Dickinson, Heidelberg, Germany) to remove debris and clumps of cells. Light microscopic examination of the harvest after metachromatic staining with 0·1% toluidine blue indicated that mast cells constituted $1{\cdot}5 \pm 0{\cdot}2$% of total nucleated cells for dog skin or $2{\cdot}4 \pm 1{\cdot}0$% for rat skin. The mast cell purity in each species under these experimental conditions was close to the values obtained by others (Barrett et al 1985; de Mora et al 1993). The trypan blue-exclusion test

indicated a viability of total nucleated cells of more than 90%.

In a separate experiment, rat peritoneal mast cells were obtained by direct lavage with Tyrode solution containing heparin (5 units mL^{-1}), and purified by density gradient centrifugation on Percoll according to Shanahan et al (1985). Cells examined under a light microscope for purity were greater than 95%, with 98% viability.

Effect of fluoroquinolones on histamine release from mast cells in-vitro

Cell suspension samples (50 μL) ($2-5 \times 10^4$ mast cells in each tube for dispersed skin cells or 1×10^5 mast cells in each tube for rat peritoneal cells) in duplicate were equilibrated at 37°C for 10 min, and then added to polypropylene tubes containing 450 μL test compounds dissolved in Tyrode solution. The concentrations examined were $1-1000\ \mu g\ mL^{-1}$ for canine mast cells and $10-1000\ \mu g\ mL^{-1}$ for rat mast cells. Histamine release reaction was allowed to proceed for a further 30 min unless otherwise indicated, at the end of which time the reaction was terminated by adding 2 mL ice-cold Tyrode solution and the mixture was centrifuged (450 *g* for 5 min at 4°C). For determination of total histamine content, mast cells were disrupted in boiling water for 10 min. Histamine concentrations in the resulting supernatant and disrupted cells were determined by the enzyme immunoassay described above. Histamine release was expressed as a percentage of total histamine content. Spontaneous histamine release from each mast cell was $5{\cdot}7 \pm 1{\cdot}8\%$ for canine skin mast cells, $6{\cdot}0 \pm 1{\cdot}0\%$ for rat skin mast cells, and $1{\cdot}8 \pm 0{\cdot}7\%$ for rat peritoneal mast cells.

Lactate dehydrogenase release was assayed using a lactate dehydrogenase test kit (MTX "LDH", Kyokuto Pharmaceutical Co., Tokyo, Japan), to determine if histamine release by fluoroquinolones was the result of nonspecific cell membrane damage (Lagunoff & Martin 1983). The lactate dehydrogenase release was expressed as a percentage of total lactate dehydrogenase release occurring in the presence of 0·1% Triton X-100.

Effects of temperature and energy on fluoroquinolone-induced histamine release from canine skin mast cells

A histamine release assay was conducted following pretreatment of cells for 30 min at 47°C or preincubation with antimycin A (1 μM) and 2-deoxy-D-glucose (2 mM) for 30 min at 37°C as described by Shanahan et al (1985). The concentrations of fluoroquinolones or compound 48/80 were fixed to be $100\ \mu g\ mL^{-1}$ or $1\ \mu g\ mL^{-1}$, respectively, which were the same order of potency observed for histamine release from canine skin mast cells.

Statistical analysis

The data are expressed as the mean ± s.e.m. Statistical analysis was carried out using unpaired Student's *t*-test or Dunnett's multiple comparison test; $P < 0{\cdot}05$ was regarded as significant.

Results

Effects of fluoroquinolones on plasma histamine concentration in-vivo

Intravenous injection of levofloxacin or ciprofloxacin at $1-10\ mg\ kg^{-1}$ produced a marked elevation in plasma histamine concentration in anaesthetized dogs in a dose-dependent manner, with a significant increase at $10\ mg\ kg^{-1}$. The plasma concentrations of histamine in dogs given levofloxacin at $10\ mg\ kg^{-1}$ were approximately one-half that of ciprofloxacin at $10\ mg\ kg^{-1}$; however, statistical analysis revealed no significant difference between the levofloxacin- and ciprofloxacin-treated groups (Table 1).

Intravenous injection of levofloxacin in anaesthetized rats at doses up to $100\ mg\ kg^{-1}$ caused little or no increase in plasma histamine concentration, whereas ciprofloxacin at $100\ mg\ kg^{-1}$ caused a significant elevation in plasma histamine levels to $845{\cdot}9 \pm 71{\cdot}0\ ng\ mL^{-1}$ (Table 2). Compound 48/80 at a dose of $0{\cdot}1\ mg\ kg^{-1}$ caused sig-

Table 1. Effects of levofloxacin, ciprofloxacin and compound 48/80 on plasma histamine concentration in anaesthetized dogs.

Test compounds	Concn ($mg\ mL^{-1}$)	Plasma histamine concn ($ng\ mL^{-1}$)
Control (saline)	0	$0{\cdot}5 \pm 0{\cdot}1$
Levofloxacin	0·3	$0{\cdot}4 \pm 0{\cdot}1$
	1	$0{\cdot}9 \pm 0{\cdot}4$
	3	$55{\cdot}8 \pm 20{\cdot}3$
	10	$420{\cdot}7 \pm 107{\cdot}7$*
Ciprofloxacin	0·3	$0{\cdot}4 \pm 0{\cdot}1$
	1	$16{\cdot}7 \pm 10{\cdot}7$
	3	$301{\cdot}8 \pm 121{\cdot}8$
	10	$957{\cdot}5 \pm 365{\cdot}6$**
Compound 48/80	0·1	$214{\cdot}7 \pm 80{\cdot}6$††

Plasma histamine concentrations were assessed 5 min after the administration of the test compounds. Values represent the mean ± s.e.m. of four to five animals. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared with the control group (Dunnett's test). ††$P < 0{\cdot}01$ compared with the control group (Student's *t*-test).

Table 2. Effects of levofloxacin, ciprofloxacin and compound 48/80 on plasma histamine concentration in anaesthetized rats.

Test compounds	Concn (mg mL^{-1})	Plasma histamine concn (ng mL^{-1})
Control (saline)	0	24·3 ± 7·3
Levofloxacin	10	22·7 ± 2·1
	30	20·9 ± 2·9
	100	20·0 ± 2·6
Ciprofloxacin	10	20·0 ± 2·6
	30	40·3 ± 9·4
	100	845·9 ± 71·0**‡
Compound 48/80	0·1	221·0 ± 134·5††

Plasma histamine concentrations were assessed 5 min after the administration of the test compounds. Values represent the mean ± s.e.m. of five animals. $^{**}P < 0.01$ compared with the control group (Dunnett's test). $\dagger\dagger P < 0.01$ compared with the control group (Student's *t*-test). $\ddagger P < 0.01$ compared with the levofloxacin-treated group (Student's *t*-test).

nificant histamine liberation in both species (Tables 1 and 2).

Effects of fluoroquinolones on histamine release from mast cells in-vitro

Levofloxacin or ciprofloxacin at concentrations ranging from 30 to 1000 μg mL^{-1} and 10 to 1000 μg mL^{-1}, respectively, caused a concentration-dependent release of histamine from canine skin mast cells (Figure 1). A significant secretion was seen at concentrations above 100 μg mL^{-1} of either fluoroquinolone. Maximum histamine release from canine skin mast cells was 24·4 ± 2·9% and 46·6 ± 3·1% at the highest concentrations of levofloxacin and ciprofloxacin, respectively.

Levofloxacin at concentrations up to 1000 μg mL^{-1} failed to induce histamine secretion from rat skin mast cells, whereas ciprofloxacin caused a significant histamine release from these cells at the highest concentration (Figure 1).

Compound 48/80 at concentrations of 0·1–10 μg mL^{-1} induced histamine secretion from dog and rat skin mast cells in a dose-dependent fashion.

The release of histamine from dispersed skin mast cells induced by fluoroquinolones was not accompanied by lactate dehydrogenase leakage in the supernatants, even at concentrations fully active in evoking histamine secretion (Figure 2).

The histamine release from canine skin mast cells induced by fluoroquinolones was rapid, being complete 15 s after fluoroquinolone addition (Figure 3).

Rat peritoneal mast cells displayed slightly greater susceptibility to the fluoroquinolones than rat skin mast cells. Levofloxacin and ciprofloxacin induced significant histamine release from rat peritoneal mast cells at concentrations of

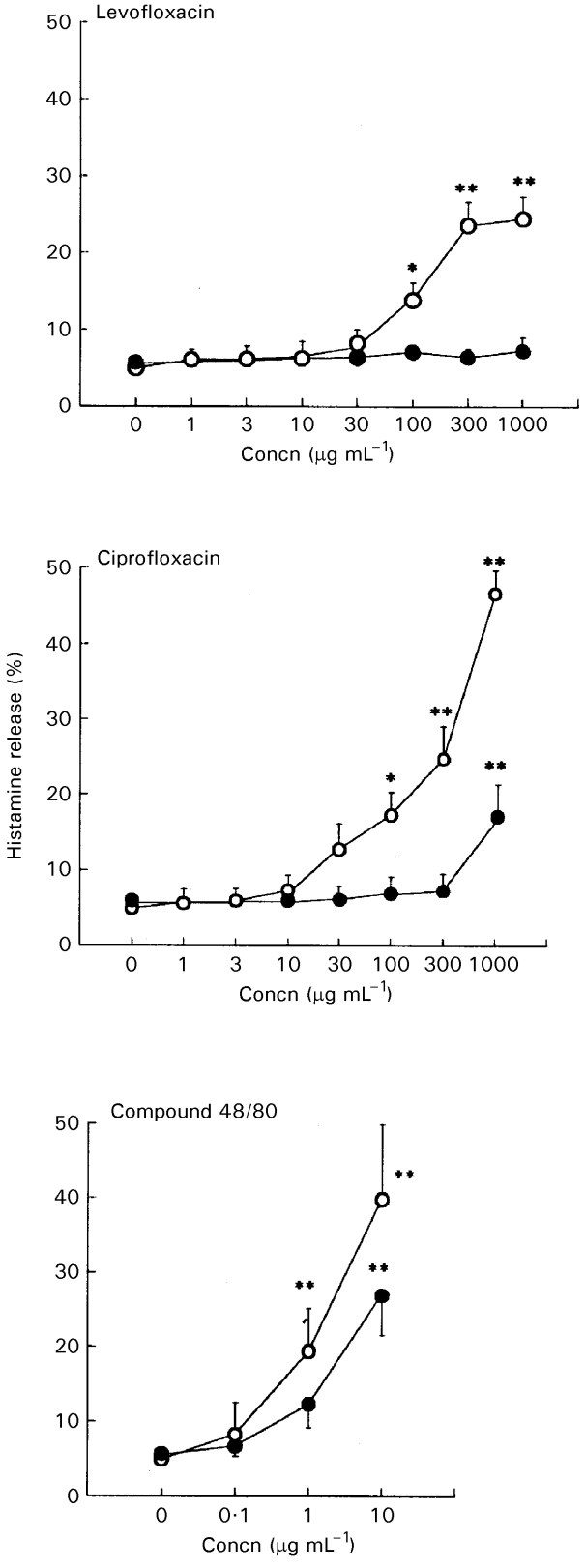

Figure 1. Effects of levofloxacin, ciprofloxacin and compound 48/80 on histamine release from canine skin mast cells (○) and rat skin mast cells (●). Histamine release was assessed after a 30-min incubation period. Values represent the mean ± s.e.m. of five separate experiments performed in duplicate. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with spontaneous histamine release (Dunnett's test).

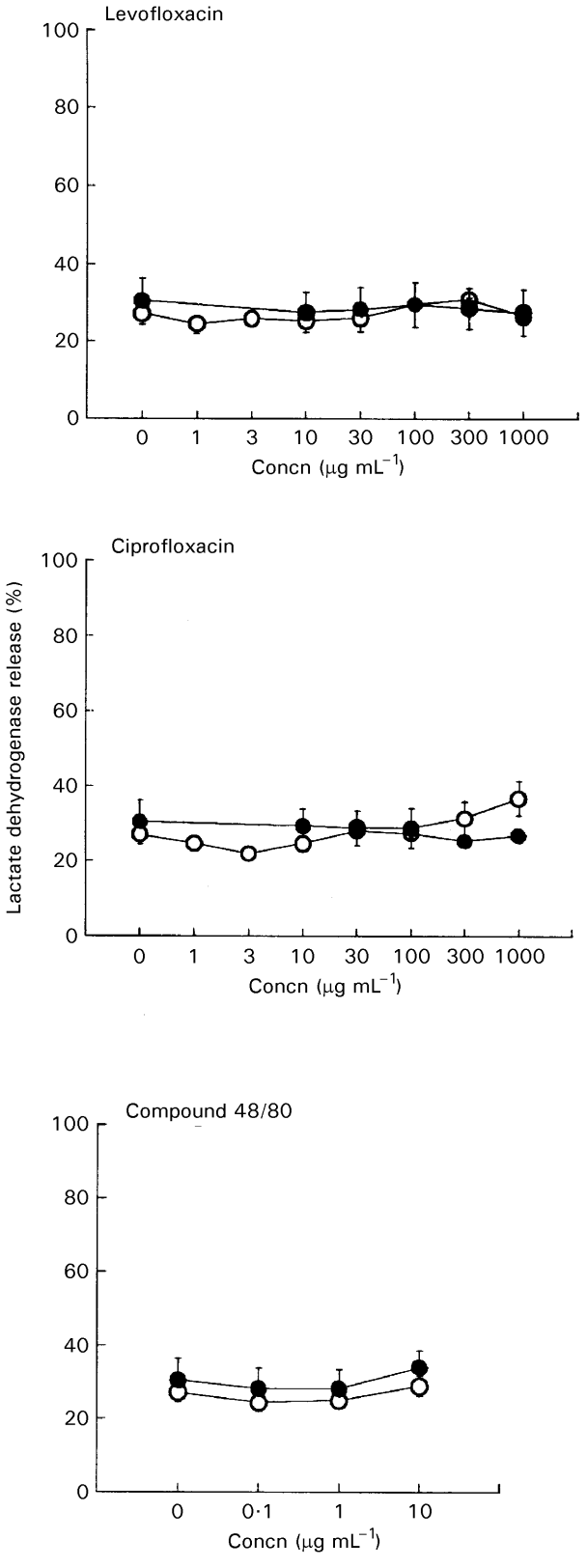

Figure 2. Effects of levofloxacin, ciprofloxacin and compound 48/80 on lactate dehydrogenase release from canine skin mast cells (○) and rat skin mast cells (●). Lactate dehydrogenase release was assessed after a 30-min incubation period. Values represent the mean ± s.e.m. of five separate experiments performed in duplicate.

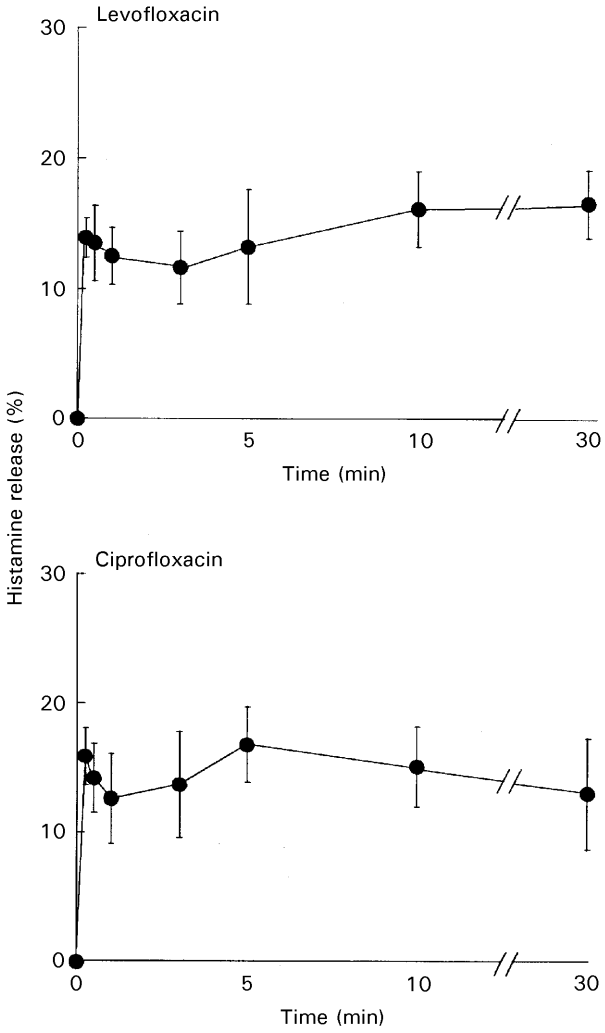

Figure 3. Kinetics of histamine release from canine skin mast cells induced by levofloxacin and ciprofloxacin. Canine skin mast cells were incubated with levofloxacin (100 μg mL^{-1}) or ciprofloxacin (100 μg mL^{-1}) for 0 to 30 min. Values represent the mean ± s.e.m. of four separate experiments performed in duplicate.

1000 μg mL^{-1} and above 300 μg mL^{-1}, respectively, with a maximum release of 10% for levofloxacin and 90% for ciprofloxacin at 1000 μg mL^{-1}. Fluoroquinolone-induced histamine release from rat peritoneal mast cells was not accompanied by lactate dehydrogenase leakage or changes in cell viability (Table 3).

Effects of temperature and energy on fluoroquinolone-induced histamine release from canine skin mast cells

Pretreatment of canine skin mast cells at 47°C for 30 min almost completely abolished fluoroquinolone-induced secretory responses. Similarly, no significant histamine secretion occurred when the reaction was conducted in a glucose-free med-

Table 3. Effects of levofloxacin and ciprofloxacin on histamine and lactate dehydrogenase release, and cell viability.

Test compounds	Concn ($\mu g\,mL^{-1}$)	Histamine (% release)	Lactate dehydrogenase (% release)	Cell viability (%)
Spontaneous (Tyrode)	0	1·8 ± 0·7	4·8 ± 1·8	97·8 ± 0·7
Levofloxacin	10	3·0 ± 0·9	4·8 ± 1·6	97·4 ± 0·7
	30	2·6 ± 0·8	4·8 ± 1·3	98·4 ± 0·4
	100	3·1 ± 1·0	4·7 ± 1·3	98·2 ± 0·5
	300	2·5 ± 0·7	5·4 ± 1·5	97·4 ± 0·7
	1000	11·2 ± 2·2**	4·6 ± 1·4	95·8 ± 0·9
Ciprofloxacin	10	3·9 ± 1·2	5·7 ± 1·7	97·8 ± 0·5
	30	2·1 ± 0·9	5·8 ± 1·9	97·2 ± 0·2
	100	1·9 ± 0·6	4·8 ± 1·2	96·8 ± 1·0
	300	43·1 ± 1·6**‡	5·8 ± 1·5	95·8 ± 0·9
	1000	87·4 ± 3·4**‡	5·9 ± 1·5	93·8 ± 0·7
Compound 48/80	1	71·0 ± 4·3††	5·2 ± 1·1	96·2 ± 0·7

Rat peritoneal mast cells were stimulated with levofloxacin or ciprofloxacin for 30 min at 37°C. Values represent the mean ± s.e.m. of five separate experiments performed in duplicate. $^{**}P < 0.01$ compared with the spontaneous release (Dunnett's test). $\dagger\dagger P < 0.01$ compared with the spontaneous release (Student's *t*-test). $\ddagger P < 0.01$ compared with the levofloxacin group at the same concentration (Student's *t*-test).

Table 4. Effects of temperature and energy on fluoroquinolone-induced histamine release from canine skin mast cells.

Test compounds	Concn ($\mu g\,mL^{-1}$)	Control[a]	47°C[b]	Antimycin A + 2-deoxy-D-glucose[c]
Spontaneous (Tyrode)	0	6·3 ± 1·8	7·3 ± 2·1	6·7 ± 2·0
Levofloxacin	100	15·0 ± 2·0*	8·2 ± 1·2††	5·6 ± 1·9††
Ciprofloxacin	100	17·3 ± 2·0**	7·0 ± 1·3††	7·0 ± 2·0††
Compound 48/80	1	15·7 ± 2·3**	6·3 ± 1·8††	6·4 ± 1·6††
Triton X-100 (%)	0·1	96·9 ± 0·5**	96·4 ± 12·6**	94·5 ± 5·0**

Canine skin mast cells were pre-incubated for 30 min at 37°C in the absence (a) or presence (c) of antimycin A (1 μM) and 2-deoxy-D-glucose (2 mM). Canine skin mast cells were pre-incubated for 30 min at 47°C (b). After washing, cells were stimulated with the test compounds for 10 min at 37°C. Values represent the mean ± s.e.m. of four to five separate experiments performed in duplicate. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the spontaneous release (Student's *t*-test). $\dagger\dagger P < 0.01$ compared with the control group (Student's *t*-test).

ium following pre-incubation with antimycin A (1 μM) and 2-deoxy-D-glucose (2 mM) for 30 min. These manipulations almost completely blocked compound 48/80-induced mast cell degranulation, but did not influence the cytolytic effects of Triton X-100 (Table 4).

Discussion

We have characterized some of the functional heterogeneity between dogs and rats, in histamine-releasing activity of fluoroquinolones. Intravenous levofloxacin or ciprofloxacin evoked a marked elevation in plasma histamine concentration in anaesthetized dogs, but had limited or negligible effect in anaesthetized rats. The results indicated that there was species specificity in the histamine-releasing activity of fluoroquinolones, and that the dog is 30- to 100-fold more susceptible to the fluoroquinolones than the rat. These findings lend support to the assumption that the inter-species difference in the hypotensive effect of levofloxacin may be related to the ability of the species to release histamine on the administration of the compound (Takasuna et al 1992).

The diversity of systemic reactions to the fluoroquinolones between these two species is almost parallel to the histamine-releasing activity of the compounds for skin mast cells in-vitro. Levofloxacin at concentrations above 30 $\mu g\,mL^{-1}$ and ciprofloxacin at 10 $\mu g\,mL^{-1}$ induced detectable histamine secretion from canine skin mast cells. We reported previously that intravenous infusions of levofloxacin or ciprofloxacin at 30 $mg\,kg^{-1}$ over 30 min produced endogenous histamine release when their plasma concentrations reached approximately 10 $\mu g\,mL^{-1}$ or more (Mori et al 1996). It would thus be possible for the presumed maximal concentrations of these compounds in skin tissue to reach a level causing histamine secretion in-vitro, since the concentration of levofloxacin in skin tissue was almost parallel to that in blood (Aoki et al 1991). Furthermore, concentrations of

levofloxacin and ciprofloxacin activating canine skin mast cells in-vitro were similar to those that induced a flush response (Kurata et al 1995) or increased cutaneous vascular permeability (Furuhata et al 1998b) after intradermal administration of several fluoroquinolones, including ofloxacin and ciprofloxacin, in the dog. The findings suggested that the elevated plasma histamine level associated with the injection of levofloxacin and ciprofloxacin in dogs appeared to be caused, in part, by liberation of histamine due to direct cutaneous mast cell activation. On the other hand, substantial evidence for the functional heterogeneity of canine mast cells from different tissues and basophils has been presented (Nishiyama et al 1957; Muldoon et al 1984; Ennis et al 1989). It should therefore be considered that histamine derived from different organs such as the liver, lung and kidney, and basophils, where very high concentrations of the compounds enter the regional circulation following intravenous dosing, may also contribute to the elevation of the plasma histamine level by fluoroquinolones.

Levofloxacin at concentrations up to $1000\,\mu g\,mL^{-1}$ failed to induce histamine release from rat skin mast cells, but ciprofloxacin induced a pronounced histamine secretion from this type of cell at the highest concentration tested. The action of fluoroquinolones on rat skin mast cells in-vitro may reflect the systemic reaction observed in-vivo, since a marked elevation in plasma histamine was detectable after intravenous injection of ciprofloxacin, but not levofloxacin, in anaesthetized rats. In contrast to rat skin mast cells, levofloxacin at the highest concentration produced a slight liberation of histamine from rat peritoneal mast cells, and ciprofloxacin induced a pronounced histamine secretion from this cell type at concentrations above $300\,\mu g\,mL^{-1}$. The reason for the difference in extent of induced histamine secretion between rat skin and peritoneal mast cells is unclear. However, the results with peritoneal mast cells indicated that levofloxacin as well as ciprofloxacin possessed substantial histamine releasing activity on mast cells of rats. This finding was supported by the observation of Yoshida et al (1994) that levofloxacin and ciprofloxacin increased the permeability of rat skin microvasculature through the induction of histamine release from mast cells.

We have attempted to elucidate the characteristics of mast cell degranulation by fluoroquinolones. The kinetics of fluoroquinolone-induced histamine secretion from canine skin mast cells were rapid enough to explain that anaphylactoid reactions to these compounds had occurred just after intravenous injection. It seems unlikely that histamine secretion induced by fluoroquinolones resulted from drug-induced cell membrane damage, since these actions were without lactate dehydrogenase leakage or changes in cell viability (Lagunoff & Martin 1983). In addition, the blockade of release by inhibition of glycolysis and oxidative phosphorylation was strongly suggestive of a non-cytotoxic, energy-requiring process (Lagunoff & Martin 1983). Non-cytotoxic histamine secretion by fluoroquinolones from several types of mast cell confirmed the observation reported by Furuhata et al (1998b) that ciprofloxacin at a concentration greater than $400\,\mu g\,mL^{-1}$ was capable of inducing histamine release from rat peritoneal mast cells, without resultant cell death.

The mechanisms whereby fluoroquinolones induced degranulation of mast cells are yet to be elucidated. Interestingly, T-3762, a new fluoroquinolone agent, substituted with a 1-aminocyclopropyl moiety at the 7 position of the quinolone ring, has little or no histamine releasing activity, even in dogs, the species most sensitive to fluoroquinolones (Furuhata et al 1998b). Many fluoroquinolones, including levofloxacin and ciprofloxacin, with a piperazinyl moiety at their 7 position retain this activity (Kojima et al 1984; Takasuna et al 1992; Kurata et al 1995; Furuhata et al 1998a, b; this study). It is therefore suggested that basic substituents at the 7 position of the quinolone ring would be responsible, in part, for histamine releasing activity of fluoroquinolones.

Species specificity for histamine releasing potency of levofloxacin and ciprofloxacin raises questions about where the compounds act as a histamine releaser in man. Nakagawa et al (1995) reported that ciprofloxacin released histamine not only from rat peritoneal mast cells but also human dispersed skin mast cells at concentrations above $200\,\mu g\,mL^{-1}$. Based on that report and these results, it is postulated that the reactivity of human mast cells to fluoroquinolones may be similar to that seen with rat mast cells rather than that with canine mast cells. This assumption is supported in part by clinical reports that most fluoroquinolones developed had little or no effects on cardiovascular systems even after intravenous applications (Thorsteinsson et al 1987, 1988; Verho et al 1988; Arcieri et al 1989; Shar & Mulert 1990; Stahlmann 1990). In contrast, several fluoroquinolones including ofloxacin and ciprofloxacin have been reported to induce cutaneous reactions, such as erythaema, burning sensation and itching mainly at the injection site in man (Thorsteinsson et al 1987, 1988; Verho et al 1988; Arcieri et al 1989). The local release of histamine and/or vasoactive sub-

stances has been suggested as being involved in these adverse reactions (Thorsteinsson et al 1987, 1988).

The above findings suggest that the functional heterogeneity of mast cells from different species may exist in histamine releasing activity of fluoroquinolones, and that mast cells from the dog appear to be particularly sensitive to the effect of fluoroquinolones.

Acknowledgements

The authors wish to thank Dr K. Furuhama, Research Planning and Administration Department, Daiichi Pharmaceutical Co., for his helpful discussion and review of this manuscript.

References

Aoki, H., Okazaki, O., Kurata, T., Shintani, S., Tachizawa, H., Hakusui, H. (1991) The pharmacokinetics of DR-3355 (I): absorption, distribution and excretion after a single oral administration to rats. Xenobio. Metab. Dispos. 6: 793–803

Arcieri, G. M., Becker, N., Esposito, B., Griffith, E., Heyd, A., Neumann, C., O'Brien, B. (1989) Safety of intravenous ciprofloxacin. A review. Am. J. Med. 87(Suppl. 5A): 92S–97S

Barrett, K. E., Ali, H., Pearce, F. L. (1985) Studies on histamine secretion from enzymatically dispersed cutaneous mast cells of the rat. J. Invest. Dermatol. 84: 22–26

de Mora, F., Garcia, G., Ferrer, L., Arboix, M. (1993) Canine cutaneous mast cells dispersion and histamine secretory characterization. Vet. Immunol. Immunopathol. 39: 421–429

Ennis, M., Amon, E. U., Lorenz, W. (1989) Histamine release from canine lung and liver mast cells induced by radiographic contrast media. Agents Actions 27: 101–103

Furuhata, K., Hayakawa, H., Soumi, K., Arai, H., Watanabe, Y., Narita, H. (1998a) Histamine-releasing properties of T-3762, a novel fluoroquinolone antimicrobial agent in intravenous use. Effects of doses and infusion rate on blood pressure, heart rate and plasma histamine concentration. Biol. Pharm. Bull. 21: 456–460

Furuhata, K., Fukuda, Y., Soumi, K., Arai, H., Watanabe, Y., Narita, H. (1998b) Histamine-releasing properties of T-3762, a novel fluoroquinolone antimicrobial agent in intravenous use. Dermovascular permeability-increasing effect and action on peritoneal mast cells. Biol. Pharm. Bull. 21: 461–464

Genovese, A., Stellato, C., Marsella, C. V., Adt, M., Marone, G. (1996) Role of mast cells, basophils and their mediators in adverse reactions to general anesthetics and radiocontrast media. Int. Arch. Allergy Immunol. 110: 13–22

Kojima, H., Hirohashi, M., Sakurai, T., Kasai, Y., Akashi, A. (1984) General pharmacology of DL-8280. Chemotherapy 32(Suppl. 1): 1148–1161

Kurata, M., Kasuga, Y., Nanbe, E., Nakamura, H., Asano, T., Haruta, K. (1995) Flush induced by fluoroquinolones in canine skin. Inflamm. Res. 44: 461–465

Lagunoff, D., Martin, T. W. (1983) Agents that release histamine from mast cells. Ann. Rev. Pharmacol. Toxicol. 23: 331–351

Lawman, M. A., Rees, P. H., Benyon, R. C., Church, M. K. (1988) Human mast cell heterogeneity: histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and nonimunologic stimuli. J. Allergy Clin. Immunol. 81: 590–597

Mori, K., Hagiwara, T., Takasuna, K., Nomura, M. (1996) Species differences in histamine release induced by fluoroquinolones. Jpn. J. Pharmacol. 71(Suppl.): 146

Muldoon, S. M., Donlon, M. A., Todd, R., Helgeson, E. A., Freas, W. (1984) Plasma histamine and hemodynamics responses following administration of nalbuphine and morphine. Agents Actions 15: 229–234

Nakagawa, T., Shimada, J., Mizushima, Y., Takaishi, T., Morita, Y. (1995) Effect of ciprofloxacin on histamine release from human and rat mast cells. Jpn. J. Inflamm. 15: 337–338

Nishiyama, R., Tasaka, K., Irino, S. (1957) The site of some histamine-releasing substances in the dog. Acta. Med. Okayama 11: 133–144

Pearce, F. L. (1982) Functional heterogeneity of mast cells from different species and tissues. Klin. Wochenschr. 60: 954–957

Pearce, F. L., Ennis, M. (1980) Isolation and some properties of mast cells from the mesentery of the rat and guinea pig. Agents Actions 10: 124–131

Schacht, P., Arcieri, G., Branolte, J., Bruck, H., Chyský, V., Griffith, E., Gruenwaldt, G., Hullmann, C., Konopka, C. A., O'Brien, B., Rahm, V., Ryoki, T., Westwood, A., Weuta, H. (1988) Worldwide clinical data on efficacy and safety of ciprofloxacin. Infection 16(Suppl.): S29–S43

Shanahan, F., Denburg, J. A., Fox, J., Bienenstock, J., Befus, D. (1985) Mast cell heterogeneity: effects of neuroenteric peptides on histamine release. J. Immunol. 135: 1331–1337

Shar, P. M., Mulert, R. (1990) Safety profile of quinolones. Eur. Urol. 17(Suppl. 1): 46–51

Smythe, M. A., Rybak, M. J. (1989) Ofloxacin: A review. Drug Intell. Clin. Pharm. Ann. Pharmacother. 23: 839–846

Stahlmann, R. (1990) Safety profile of the quinolones. J. Antimicrob. Chemother. 26(Suppl. D): 31–44

Takasuna, K., Kasai, K., Usui, C., Takahashi, M., Hirohashi, M., Tamura, K., Takayama, S. (1992) General pharmacology of the new quinolone antibacterial agent levofloxacin. Arzneim. Forsch. Drug. Res. 42: 408–418

Thorsteinsson, S. B., Bergan, T., Johannesson, G., Thorsteinsson, H. S., Rohwedderm, R. (1987) Tolerance of ciprofloxacin at injection site, systemic safety and effect on electroencephalogram. Chemotherapy 33: 448–451

Thorsteinsson, S. B., Bergan, T., Rohwedder, R. (1988) Tolerance of intravenously administered ciprofloxacin. Chemotherapy 34: 256–260

Une, T., Fujimoto, T., Sato, K., Osada, Y. (1988) In vitro activity of DR-3355, an optically active ofloxacin. Antimicrob. Agents Chemother. 32: 1336–1340

Verho, M., Malerczyk, V., Grötsch, H., Lorenz, H. (1988) Absence of crystalluria and estimation of renal parameters after oral and intravenous ofloxacin as compared to placebo in healthy volunteers. Drug Exp. Clin. Res. 14: 539–545

Yoshida, M., Takayama, S., Kato, M. (1994) Effect of levofloxacin and ciprofloxacin injection on permeability of the tail vein in mice and skin microvasculature in rats. Int. J. Tissue React. 16: 105–112