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Short communication

Mechanism of histamine release induced by levofloxacin, a fluoroquinolone antibacterial agent

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Abstract

The present study was designed to clarify the mechanism of histamine release caused by levofloxacin, a fluoroquinolone antibacterial agent, using rat peritoneal mast cells. Levofloxacin induced a concentration-dependent histamine secretion from 300 μg/ml without lactate dehydrogenase leakage, and the release was rapidly completed within 30 s. This action was dependent on temperature, energy, pH and intracellular Ca^{2+}, similarly to the effect of compound 48/80, a basic compound. Unlike that with the calcium ionophore A23187, histamine secretion due to levofloxacin or compound 48/80 was prevented by pretreatment with either pertussis toxin or benzalkonium chloride, a selective inhibitor of G proteins of G_i subtypes. Moreover, the histamine release elicited by levofloxacin or compound 48/80 was suppressed by hydrolysis of sialic acid residues on the cell surface brought about by neuraminidase. These results demonstrate that the mechanism by which levofloxacin exerts histamine release may be closely linked to activation of pertussis toxin-sensitive G proteins.

Keywords: Fluoroquinolone; Histamine release; Mast cell; G protein

1. Introduction

The new fluoroquinolone antibacterial agents such as levofloxacin, ofloxacin, and ciprofloxacin are widely used for the treatment of various infections, because of their broad antibacterial spectra, bactericidal activities and good pharmacokinetics (Schacht et al., 1988; Une et al., 1988; Smyth and Rybak, 1989). Among the fluoroquinolones, levofloxacin, ofloxacin, and ciprofloxacin have been shown to cause endogenous histamine release in dogs and rats following intravenous or intradermal injection (Takasuna et al., 1992; Yoshida et al., 1994; Kurata et al., 1995; Furuhata et al., 1998a,b; Mori et al., 2000), with dogs being particularly sensitive to these fluoroquinolones (Furuhata et al., 1998a,b; Mori et al., 2000). In humans, ofloxacin or ciprofloxacin injection was reported to induce cutaneous reactions, such as erythema, burning sensation and itching, mainly at the administration site (Thorsteinsson et al., 1987, 1988; Verho et al., 1988; Arcieri et al., 1989). In certain cases of these adverse reactions, the local release of histamine and/or vasoactive substances was suggested to occur (Thorsteinsson et al., 1987, 1988). We previously reported that histamine release induced by levofloxacin or ciprofloxacin from canine skin mast cells, and from rat skin and peritoneal mast cells was not due to cytotoxicity (Mori et al., 2000). In the present study, using rat peritoneal mast cells, we investigated the mechanism of histamine release induced by levofloxacin. These cells were considered to be appropriate ones for the purpose of the present investigation, since the sensitivity of this cell type to fluoroquinolones resembles that of human skin mast cells more than do canine mast cells (Nakagawa et al., 1995; Mori et al., 2000).

2. Materials and methods

2.1. Chemicals

Levofloxacin used in the present study was synthesized at Daiichi Pharmaceutical. Compound 48/80, calcium

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ionophore A23187, bovine serum albumin, 2-deoxy-D-glucose, antimycin A, pertussis toxin, benzalkonium chloride, neuraminidase (type V, from *Clostridium perfringens*), and Triton X-100 were purchased from Sigma (St. Louis, MO, USA). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Levofloxacin was dissolved in modified Tyrode's solution (pH 7.4) containing 124 mM NaCl, 4 mM KCl, 0.64 mM NaH_2PO_4, 10 mM $NaHCO_3$, 1.6 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 0.05% bovine serum albumin, and the pH of the solution was adjusted to 7.4 with NaOH. All reagents utilized were analytical grade.

2.2. Cell preparations

The experimental protocols were in accordance with our institutional guidelines for use of laboratory animals. Peritoneal mast cells from male Sprague–Dawley rats weighing 250–400 g (Japan SLC, Hamamatsu, Japan) were obtained by direct lavage with Tyrode's solution containing heparin (5 U/ml), and purified by density-gradient centrifugation with Percoll as described previously (Mori et al., 2000). The purity of cells, examined under a light microscope, was higher than 95% with 98% viability.

2.3. Histamine release assay

Aliquots of 450 μl of cell suspension (1×10^5mast cells/tube) in duplicate were equilibrated at 37°C for 10 min, and then incubated in a polypropylene tube containing 50 μl of the test solution. The 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 the addition of 2 ml ice-cold Tyrode's solution, and the mixture was centrifuged (450 × *g* at 4°C for 5 min). For determination of total histamine content, mast cells were disrupted in boiling water for 10 min. Histamine concentrations in the resulting supernatant or in disrupted cells were determined with a commercially available ELISA kit (Histamine-ELISA, ICN Pharmaceutical, CA, USA). None of the test compounds used interfered with the ELISA method. Histamine release was expressed as a percentage of total histamine content. All values were corrected for the spontaneous release occurring in the absence of the secretagogues.

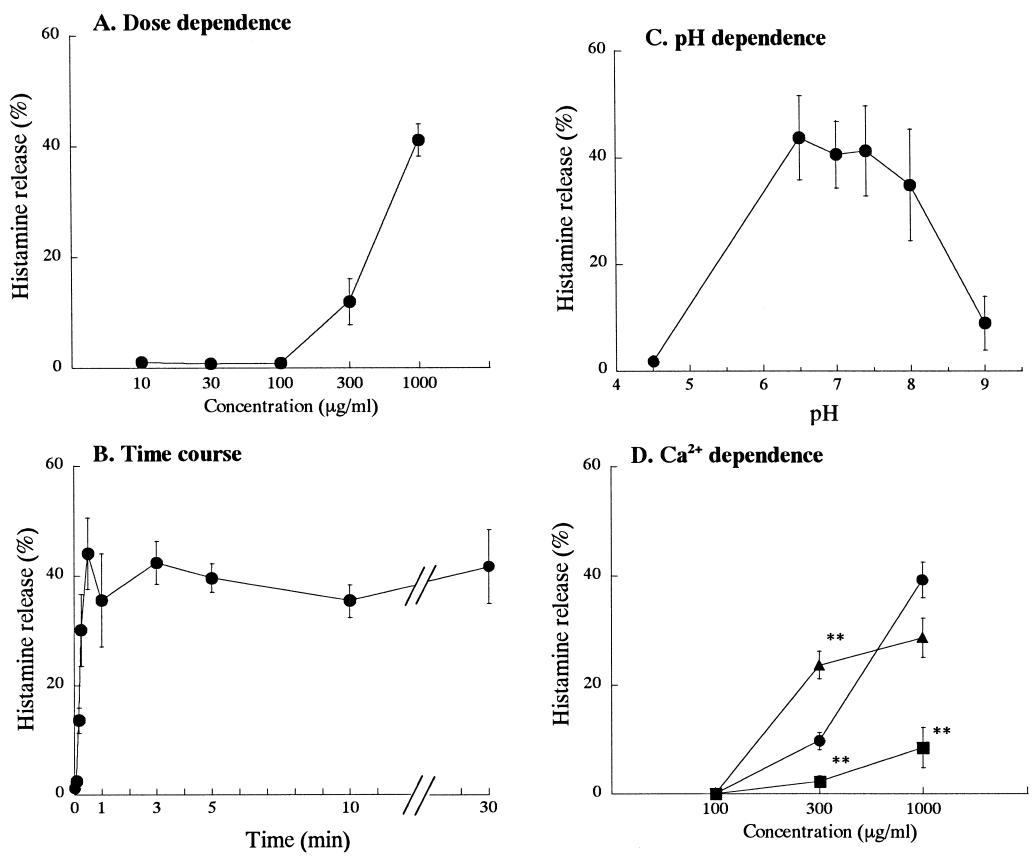

Fig. 1. Histamine release induced by levofloxacin (LVFX) from rat peritoneal mast cells. (A) Concentration dependence of histamine release. (B) Time course of histamine release induced by LVFX (1000 μg/ml). (C) pH dependence of histamine release induced by LVFX (1000 μg/ml) in the incubation medium ranging from pH 4.5 to 9. (D) Ca^{2+} dependence of histamine release. Histamine release assay was performed in the presence (●) or absence (▲) of calcium in Tyrode's solution, or after pre-incubation with EDTA (■) for 120 min. Values represent the means ± S.E.M. of four separate experiments performed in duplicate. $^{**}P < 0.01$: compared with histamine release in the presence of calcium (Student's *t*-test).

To determine if histamine release induced by levofloxacin results from non-specific cell membrane damage (Lagunoff et al., 1983), the concentration of lactate dehydrogenase (LDH) released into the culture medium was assayed using an LDH test kit (MTX ''LDH'', Kyokuto Pharmaceutical, Tokyo, Japan). LDH leakage from the cells was measured in the same manner as described for the histamine release assay. The LDH release was expressed as a percentage of that occurring in the presence of 0.1% Triton X-100.

2.4. Effects of temperature, energy, pH, and extra- or intracellular Ca^{2+} on levofloxacin-induced histamine release

To determine whether levofloxacin-induced histamine release depends on temperature or energy, the histamine release assay was done at 4°C or 37°C following pretreatment of cells at 47°C for 30 min, or in a glucose-free medium following pre-incubation with antimycin A (1 μM) and 2-deoxy-D-glucose (2 mM) for 20 min (Shanahan et al., 1985). In addition, to determine the optimal pH of the incubation medium, we prepared Tyrode's solution with a pH ranging from 4.5 to 9 (Rüegg, 1979) and measured the histamine release. Finally, to assess the role of extra- or intracellular Ca^{2+}, histamine release was determined in the presence or absence of Ca^{2+} (1.6 mM) in the incubation medium, or following pretreatment of cells with EDTA (1 mM) for 120 min (Shanahan et al., 1985).

2.5. Effects of pertussis toxin, benzalkonium chloride, and neuraminidase on levofloxacin-induced histamine release

Mast cells were pretreated with pertussis toxin for 120 min (Nakamura and Ui, 1985) or with neuraminidase, which cleaves the sialic acid residues from membrane glycoproteins and gangliosides, for 90 min (Coleman et al., 1986; Mousli et al., 1989). After washing, the cells were stimulated with levofloxacin, compound 48/80 or calcium ionophore A23187. Mast cells were pretreated with benzalkonium chloride, a selective inhibitor of G proteins of G_i subtypes (Fischer et al., 1993) for 10 min before the addition of levofloxacin, compound 48/80 or calcium ionophore A23187 to the incubation medium (Emadi-Khiav et al., 1995).

2.6. Statistical analysis

The data are expressed as the means ± S.E.M. The statistical analysis was done with the unpaired Student's *t*-test or Dunnett's multiple comparison. A P value less than 0.05 was regarded as significant.

3. Results

3.1. Levofloxacin-induced histamine release from rat peritoneal mast cells

Levofloxacin at concentrations of 300 μg/ml (830 μM) or more caused a concentration-dependent release of histamine (Fig. 1A), and its action was not accompanied by LDH leakage or changes in cell viability (data not shown). This histamine release reached a peak within 30 s after levofloxacin addition (Fig. 1B).

3.2. Effects of temperature, energy, pH, and extra- or intracellular Ca^{2+} on levofloxacin-induced histamine release

The secretion of histamine induced by levofloxacin was completely abolished at 4°C or at 37°C following pretreatment of mast cells at 47°C for 30 min. The histamine secretion due to levofloxacin was also completely blocked

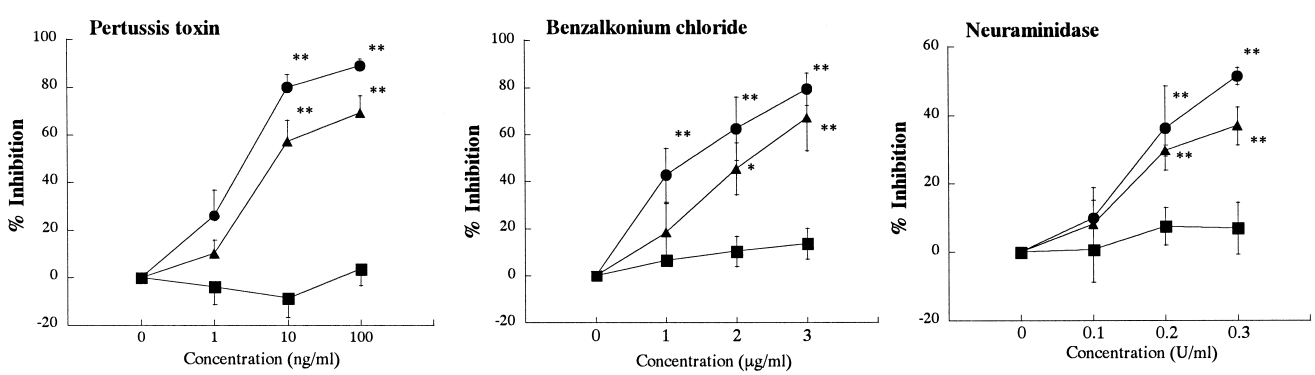

Fig. 2. Effects of pertussis toxin, benzalkonium chloride, and neuraminidase on histamine release induced by levofloxacin (LVFX, ●), compound 48/80 (▲) or calcium ionophore A23187 (■). Pretreatment of rat peritoneal mast cells with pertussis toxin or neuraminidase was performed for 120 or 90 min, respectively. After washing, the cells were stimulated with LVFX (1000 μg/ml), compound 48/80 (1 μg/ml) or calcium ionophore A23187 (1 μM). Pretreatment with benzalkonium chloride was performed for 10 min before the addition of secretagogues to the incubation medium. Values represent the means ± S.E.M. of four to five separate experiments performed in duplicate. $^{*}P < 0.05$, $^{**}P < 0.01$: compared with histamine release induced in the absence of each blocker (Dunnett's test).

in a glucose-free medium after pretreatment of the cells with antimycin A and 2-deoxy-D-glucose for 20 min. The same manipulations almost abolished compound 48/80-induced mast cell exocytosis, but did not influence the cytolytic effects of Triton X-100 (data not shown). The optimum pH for histamine release due to levofloxacin ranged from 6.5 to 7.4 (Fig. 1C).

Removal of extracellular Ca^{2+} enhanced the histamine secretion due to levofloxacin at 300 μg/ml, but had no significant effect at levofloxacin 1000 μg/ml. Depletion of intracellular Ca^{2+} after pre-incubation with EDTA for 120 min completely prevented the levofloxacin-induced secretory response at 300 μg/ml or higher (Fig. 1D).

3.3. Effects of pertussis toxin, benzalkonium chloride, and neuraminidase on levofloxacin-induced histamine release

Pretreatment of mast cells with pertussis toxin (1–100 ng/ml) for 120 min inhibited the histamine release caused by either levofloxacin (1000 μg/ml) or compound 48/80 (1 μg/ml) in a concentration-dependent fashion. In contrast, pertussis toxin of up to 100 ng/ml failed to inhibit calcium ionophore A23187 (1 μM)-induced histamine release. Benzalkonium chloride (1–3 μg/ml) concentration dependently prevented the histamine release induced by either levofloxacin (1000 μg/ml) or compound 48/80 (1 μg/ml), but had no effect on calcium ionophore A23187 (1 μM)-induced histamine release. Pre-incubation of mast cells with neuraminidase (0.1–0.3 U/ml) for 90 min suppressed histamine secretion induced by either levofloxacin (1000 μg/ml) or compound 48/80 (1 μg/ml) in a concentration-dependent manner. The histamine secretion stimulated by calcium ionophore A23187 (1 μM) was unaffected by neuraminidase (Fig. 2).

4. Discussion

We investigated the mechanism of histamine release induced by the fluoroquinolone antibacterial agent, levofloxacin, using rat peritoneal mast cells. Levofloxacin at 300 μg/ml (830 μM) or more induced a concentration-dependent histamine secretion in this test system. According to the pharmacokinetics data from healthy volunteers treated orally with a maximum clinical dose (500 mg/day) of levofloxacin, the maximum concentration (C_{max}) was about 5 μg/ml (about 14 μM) (Chien et al., 1997). The degranulation of mast cells due to levofloxacin was very rapid, reaching a maximum within 30 s, as had already been shown for compound 48/80 and substance P (Rüegg, 1979; Shanahan et al., 1985; Mousli et al., 1989). The action of levofloxacin on mast cells was considered to be non-cytotoxic, since the secretion occurred without LDH leakage or changes in cell viability. These findings are in agreement with previous observations (Furuhata et al., 1998a; Mori et al., 2000). The secretion of histamine caused by levofloxacin was dependent on temperature, indicating the involvement of an enzymatic process. In addition, the blockade of release by inhibition of glycolysis and oxidative phosphorylation was strongly suggestive of a non-cytotoxic action (Lagunoff et al., 1983). The optimum pH for histamine release due to levofloxacin ranged from 6.5 to 7.4, similar to the range for immunological and basic compounds including compound 48/80 and substance P (Bach et al., 1971; Rüegg, 1979; Shanahan et al., 1985).

Calcium is required in the stimulus-secretion coupling of the secretory system of histamine. Levofloxacin-induced histamine release occurred in the absence of extracellular Ca^{2+}, although this action was closely related to the relative concentrations of the compound. This finding is consistent with what was reported for compound 48/80 and peptide 401 by Atkinson et al. (1979). In contrast, depletion of intracellular Ca^{2+} clearly inhibited levofloxacin-induced histamine secretion, suggesting that histamine may be actively secreted through the mobilization of intracellular Ca^{2+} triggered by levofloxacin.

G proteins have been proposed as targets for various basic secretagogues such as natural and synthetic polyamines (spermine and compound 48/80), venom peptides (mastoparan), neuropeptides (substance P), and peptide hormones (bradykinin) in mast cell activation (Mousli et al., 1989, 1990a,b; Aridor et al., 1990; Bueb et al., 1990, 1992; Higashijima et al., 1990). These issues have been well-reviewed (Mousli et al., 1990b; Lorenz et al., 1998). The mast cell activation caused by either levofloxacin or compound 48/80 was completely abolished by pretreatment with pertussis toxin, unlike activation caused by the calcium ionophore A23187, implying that a pertussis toxin-sensitive G protein plays a crucial role in the levofloxacin-induced exocytosis pathway. Benzalkonium chloride blocked mast cell activation, suggesting that levofloxacin activates mast cell G proteins of the G_i subtypes such as G_{i3}, which was shown to be largely involved in the activation of mast cells (Aridor et al., 1993; Fischer et al., 1993). Moreover, binding to sialic acid residues on the cell surface appeared to be a prerequisite for the levofloxacin and G protein interaction, because levofloxacin-induced histamine release was suppressed by the hydrolysis of sialic acid residues by neuraminidase. These results demonstrate that levofloxacin interacts with sialic acid residues, followed by the activation of pertussis toxin-sensitive G proteins leading to histamine release.

Interestingly, according to a recent report (Furuhata et al., 1998a,b), the new fluoroquinolone agent, T-3762, substituted with a cyclopropyl moiety at the 7 position of the quinolone ring, which is the same structure as levofloxacin, showed little or no histamine-releasing activity, even in dogs, the animals most sensitive to fluoroquinolones. Levofloxacin, ofloxacin or ciprofloxacin are zwitterions at physiological pH, and possesses a positively charged piperazinyl moiety at the 7 position. It is therefore

assumed that this cationic moiety of levofloxacin may interact with negatively charged surface sialic acid residues, leading to mast cell activation. Further investigation is needed to define the structure–release relationship for histamine in mast cell activation induced by fluoroquinolones.

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