J. Pharm. Pharmacol. 2000, 52: 327–331
Received August 17, 1999
Accepted November 8, 1999

Histamine Release Induced by Antimicrobial Agents and Effects of Antimicrobial Agents on Vancomycin-induced Histamine Release from Rat Peritoneal Mast Cells

TEIKO TOYOGUCHI, MITSUTAKA EBIHARA, FUMIYOSHI OJIMA, JUN HOSOYA, TOHRU SHOJI AND YOSHITO NAKAGAWA

Department of Pharmacy, Yamagata University Hospital, 2-2-2 Iida Nishi, Yamagata 990-9585, Japan

Abstract

Vancomycin and certain fungicides may cause anaphylactoid reactions. We investigated the effects of vancomycin, miconazole and fluconazole on histamine release in rat peritoneal mast cells. Vancomycin and miconazole provoked histamine release in a dose-dependent manner. In contrast, fluconazole did not provoke histamine release at concentrations of 3×10^{-6}–3×10^{-3} M.

Vancomycin is efficacious in the treatment of Gram-positive bacterial infections; patients presenting themselves with mixed infections require concomitant therapy with a second antimicrobial agent. We investigated the effect of fosfomycin sodium, cilastatin sodium or fluconazole on vancomycin-induced histamine release. Fosfomycin sodium inhibited vancomycin-induced histamine release but neither cilastatin sodium nor fluconazole inhibited it in the mole ratios of daily doses used in humans.

These results suggest that vancomycin and miconazole provoke histamine release in rat mast cells, but that fluconazole probably does not, while fosfomycin sodium may inhibit vancomycin-induced histamine release.

The rapid intravenous administration of the glycopeptide antibiotic, vancomycin, may cause anaphylactoid reactions including hypotension, flushing and shortness of breath (Sahai et al 1989; Wallace et al 1991). Systemic allergic reactions and cardiac arrhythmias may occur with intravenous injection of the imidazole fungicide, miconazole (Heel et al 1980), and a rash may occur with the triazole fungicide, fluconazole. Therefore, the direct effects of vancomycin, miconazole and fluconazole on histamine release were investigated in rat peritoneal mast cells.

Many patients with serious infections have complex infections typically consisting of methicillin-resistant *Staphylococcus aureus* (MRSA), other Gram-negative bacteria and fungi. Vancomycin has a tremendous antibacterial action against Gram-positive bacteria, especially MRSA. However, concomitant therapy with a second antimicrobial agent proven to be reliably effective against other Gram-negative bacteria and fungi is frequently required in such patients. Vancomycin has the adverse reaction of nephrotoxicity. We previously reported that the nephrotoxicity associated with vancomycin was attenuated by fosfomycin sodium, cilastatin sodium or miconazole, but not by fluconazole in rabbits (Toyoguchi & Nakagawa 1996; Toyoguchi et al 1997, 1998). We were also apprehensive about using vancomycin with other antimicrobial agents which might increase the histamine release.

In the present study, the effects of these antimicrobial agents on vancomycin-induced histamine release were examined in rat peritoneal mast cells.

Correspondence: T. Toyoguchi, Department of Pharmacy, Yamagata University Hospital, 2-2-2 Iida Nishi, Yamagata 990-9585, Japan.

Materials and Methods

Reagents

Fosfomycin sodium (Meiji Pharmaceutical Co. Ltd, Tokyo, Japan), cilastatin sodium (Banyu Pharmaceutical Co. Ltd, Tokyo, Japan), miconazole (Mochida Pharmaceutical Co. Ltd, Tokyo, Japan) and fluconazole (Pfizer Pharmaceutical Co. Ltd, Tokyo, Japan) were obtained. Vancomycin was purchased from Shionogi & Co. Ltd (Osaka, Japan)

and bovine serum albumin from Wako Pure Chemical Industries, Ltd (Osaka, Japan). The other reagents were the highest grade of commercially available products.

Rat peritoneal mast cell preparations

Male Wistar rats (Clea Japan, Inc., Tokyo, Japan), 230–330 g, were anaesthetized with diethylether and exsanguinated by cutting one of the carotid arteries. The rats were immediately injected intraperitoneally with 15 mL of a phosphate-buffer solution (PBS; 150 mM NaCl, 3·7 mM KCl, 3·0 mM Na_2HPO_4, 3·5 mM KH_2PO_4, 5·6 mM glucose and 0·1% bovine serum albumin, pH 6·8). The abdomen was gently massaged for 90 s, the peritoneal cavity was opened and the intraperitoneal fluid was collected. The cells were first pelleted by centrifugation (50 × *g*, 5 min, 4°C), then resuspended in PBS and purified with 31·5% bovine serum albumin (Sullivan et al 1975). The resulting cell suspensions contained approximately 10^4 mast cells per experimental well as determined by toluidine blue staining.

Histamine release studies

Mast cell suspension (0·9 mL) in a phosphate-buffer solution (150 mM NaCl, 3·7 mM KCl, 3·0 mM Na_2HPO_4, 3·5 mM KH_2PO_4, 5·6 mM glucose and 1 mM $CaCl_2$) was pre-incubated at 37°C for 5 min. A sample (0·1 mL) of the antimicrobial agent solution was added and the mixture was incubated for another 5 min at 37°C. Miconazole and fluconazole were dissolved in dimethylsulphoxide (DMSO), and the final concentration of DMSO in the assay media was 1%(v/v). In the dose–response study of miconazole or fluconazole, 10 μL of the antimicrobial agent solution was added to 0·99 mL of the mast cell suspension. After completion of the reaction, the mixture was cooled in icewater and centrifuged (1700 *g*, 10 min, 4°C) to give the cell fraction and the supernatant fraction.

Histamine assay

The histamine contents of both fractions were fluorometrically determined, using *o*-phthalaldehyde, by high-performance liquid chromatography (Tsuruta et al 1978). The chromatographic system consisted of a TOSOH SC-8010 liquid chromatograph (TOSOH, Japan), FS8020 spectrophotofluorometer, and CCPD pump. TSK gel ODS-80TM (46 mm × 15 cm, TOSOH, Japan) was used for the column. The fluorescence intensity was monitored at the emission wavelength of 450 nm with the excitation wavelength set at 350 nm. The mobile phase was a mixture of 0·2 M NaCl solution and methanol (55 : 45) adjusted to pH 3 with 0·1 M HCl. The correlation coefficient of the calibration line was 0·996. Histamine release was expressed as a percentage of histamine in the supernatant to the total histamine content (intracellular + supernatant histamine).

Statistical analyses

All data are expressed as the mean ± s.d. Differences between groups were tested for statistical significance by the multiple comparison test (Dunnett's test). A P value of $< 0{\cdot}05$ was defined as indicating a significant difference.

Results

Vancomycin-, miconazole- or fluconazole-induced histamine release

When the rat isolated peritoneal mast cells were treated with vancomycin, histamine was released in a dose-dependent manner at a concentration of 3×10^{-4}–10^{-2} M (Figure 1). Miconazole also provoked histamine release in a dose-dependent manner at 3×10^{-6}–3×10^{-4} M (Figure 2). In contrast, fluconazole did not provoke histamine release in the 3×10^{-6}–3×10^{-3} M concentration range (Figure 2). The spontaneous histamine release was less than 5%.

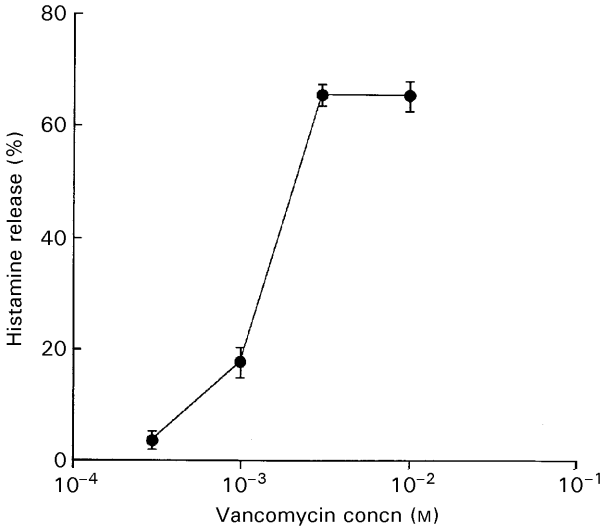

Figure 1. Histamine release by vancomycin in rat isolated peritoneal mast cells. Spontaneous histamine release was less than 5%. Each point represents the mean ± s.d. of 4 experiments.

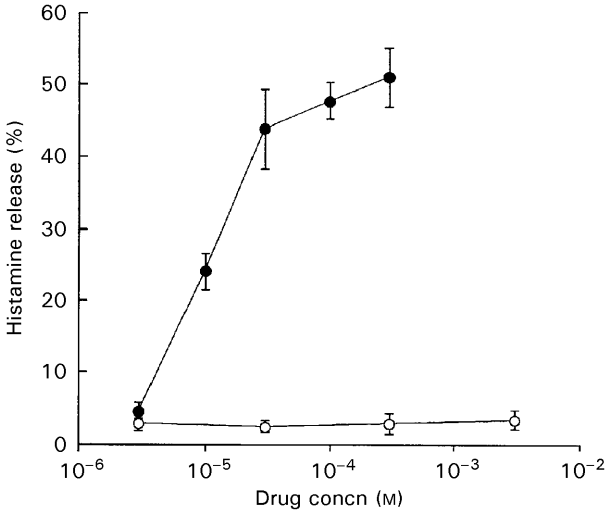

Figure 2. Histamine release by miconazole (●) and fluconazole (○) in rat isolated peritoneal mast cells. Spontaneous histamine release was less than 5%. Each point represents the mean ± s.d. of 5 experiments.

Effects of fosfomycin sodium, cilastatin sodium or fluconazole on vancomycin-induced histamine release

The daily doses in humans, of the drugs used in this study are as follows: vancomycin 2 g; fosfomycin sodium 2–4 g; cilastatin sodium 0·5–2 g; and fluconazole 50–400 mg. Therefore, the mole ratio of the vancomycin daily dose to the fosfomycin sodium daily dose is approximately 1 : 8–1 : 16, the mole ratio of vancomycin to cilastatin sodium is 1 : 1–1 : 4, and that of vancomycin to fluconazole is 1 : 0·1–1 : 1. We investigated the vancomycin-induced histamine release from rat mast cells using these mole ratios. The concentration of vancomycin was 3×10^{-3} M.

Fosfomycin sodium inhibited vancomycin-induced histamine release in a dose-dependent manner (Table 1). Cilastatin sodium did not inhibit vancomycin-induced histamine release in the mole ratio 1 : 1, but inhibited the histamine release at a ratio of 1 : 5, which was greater than the mole ratio of the vancomycin daily dose to the cilastatin sodium daily dose (Table 2).

Fluconazole did not inhibit vancomycin-induced histamine release at the mole ratio of 1 : 0·1 or 1 : 1 (Table 3).

Table 1. Effect of fosfomycin sodium on vancomycin-induced histamine release from rat isolated peritoneal mast cells.

Agent	Total % of histamine released
Control	4·55 ± 1·68*
Vancomycin	61·26 ± 1·86
Fosfomycin sodium	4·01 ± 1·46*
Vancomycin + fosfomycin sodium, 1 : 1	58·48 ± 1·32
Vancomycin + fosfomycin sodium, 1 : 5	49·82 ± 5·25*
Vancomycin + fosfomycin sodium, 1 : 10	36·42 ± 4·55*
Vancomycin + fosfomycin sodium, 1 : 20	12·68 ± 2·37*

Concentrations of vancomycin and fosfomycin sodium were 3×10^{-3} M and 6×10^{-2} M, respectively. 1 : 1, 1 : 5, 1 : 10 or 1 : 20 indicates the mole ratios of vancomycin to fosfomycin sodium. Values represent the mean ± s.d. of 4 experiments. *$P < 0.01$ vs vancomycin group.

Table 2. Effect of cilastatin sodium on vancomycin-induced histamine release from rat isolated peritoneal mast cells.

Agent	Total % of histamine released
Control	4·28 ± 0·68*
Vancomycin	65·41 ± 6·29
Cilastatin sodium	2·41 ± 0·49*
Vancomycin + cilastatin sodium, 1 : 1	61·80 ± 5·12
Vancomycin + cilastatin sodium, 1 : 5	56·05 ± 5·18*

Concentrations of vancomycin and cilastatin sodium were 3×10^{-3} M and $1{\cdot}5 \times 10^{-2}$ M, respectively. 1 : 1 or 1 : 5 indicates the mole ratios of vancomycin to cilastatin sodium. Values represent the mean ± s.d. of 5 experiments. *$P < 0.01$ vs vancomycin group.

Table 3. Effect of fluconazole on vancomycin-induced histamine release from rat isolated peritoneal mast cells.

Agent	Total % of histamine released
Control	4·14 ± 1·92*
Vancomycin	50·77 ± 5·79
Fluconazole	2·44 ± 0·70*
Vancomycin + fluconazole, 1 : 0·1	50·94 ± 1·69
Vancomycin + fluconazole, 1 : 1	53·07 ± 6·45

Concentrations of vancomycin and fluconazole were 3×10^{-3} M. 1 : 0·1 or 1 : 1 indicates the mole ratios of vancomycin to fluconazole. Values represent the mean ± s.d. of 4 experiments. *$P < 0.01$ vs vancomycin group.

Discussion

It has been reported that vancomycin caused anaphylactoid reactions in patients with high frequency (Polk 1991), and that the severity of the reaction correlated with the amount of histamine released into plasma (Wallace et al 1991). Pretreatment with histamine antagonists protected against vancomycin-induced red man syndrome (Levy et al 1987; Sahai et al 1989; Wallace et al 1991). Although tryptase is a marker of immunologic mast cell activation, plasma tryptase levels were unchanged during the vancomycin infusion (Renz et al 1998). Also, vancomycin caused release of histamine from isolated mast cells (Williams et al 1991; Hor-

inouchi et al 1993) and from dispersed human cutaneous mast cells (Levy et al 1987). These data suggest that direct histamine release occurs with vancomycin administration, but not immunologically. A rapid and transient enhancement of inositol 1,4,5trisphosphate (IP3) production and a subsequent transient increase in intracellular Ca^{2+} concentration may contribute to the vancomycin-induced histamine release from rat peritoneal mast cells (Horinouchi et al 1993).

After a farmer used a spray application of an agricultural formulation of the antifungal imidazole compound, prochloraz, an acute asthma attack occurred, which was followed by permanent airway obstruction (Gietzen et al 1996). Imidazole fungicides, ketoconazole, prochloraz and miconazole are able to elicit histamine release from mast cells. It was thus suggested that certain imidazole fungicides provoke histamine release by non-immunological mechanisms (Gietzen et al 1996).

In the present experimental study, vancomycin and miconazole provoked histamine release from rat mast cells in a dose-dependent manner. In contrast, fluconazole did not provoke histamine release at 3×10^{-6}–3×10^{-3} M. As the daily dose of miconazole in humans is greater than that of fluconazole, fluconazole is suggested to provoke less histamine release than miconazole.

Though vancomycin is highly efficacious in the treatment of Gram-positive bacterial infections, patients presenting with mixed infections require concomitant therapy with a second antimicrobial agent. We are apprehensive about using vancomycin with other antimicrobial agents which might increase histamine release. We have already reported that nephrotoxicity associated with vancomycin was attenuated by fosfomycin sodium (the mole ratio of the vancomycin dose to the fosfomycin sodium dose was 1 : 8), cilastatin sodium (the mole ratio of vancomycin to cilastatin sodium was 1 : 1, 1 : 2 or 1 : 4) or miconazole (the mole ratio of vancomycin to miconazole was 1 : 0·35), but not by fluconazole (the mole ratio of vancomycin to fluconazole was 1 : 0·5) in rabbits (Toyoguchi et al 1996, 1997, 1998). The mechanism of attenuation of nephrotoxicity remains unclear. The protective effects of these antimicrobial agents against vancomycin-induced nephrotoxicity might be partly due to the change in renal handling of vancomycin, probably in its tubular secretion/reabsorption. Another possible mechanism is that these antimicrobial agents might decrease the vancomycin-induced histamine release, with the result that vancomycin-triggered decreases in blood pressure and renal blood flow might be prevented.

In the present study, fosfomycin sodium inhibited vancomycin-induced histamine release in the mole ratio of the vancomycin daily dose to fosfomycin sodium daily dose (1 : 8–1 : 16). Cilastatin sodium did not inhibit the vancomycin-induced histamine release in the mole ratio of 1 : 1, but inhibited histamine release in the mole ratio of 1 : 5. Fluconazole did not inhibit the vancomycin-induced histamine release in the mole ratio of vancomycin daily dose to fluconazole daily dose (1 : 0·1–1 : 1). These results might almost be consistent with the data of the attenuating effects of these antimicrobial agents on the vancomycin-induced nephrotoxicity in rabbits. However, although miconazole provoked histamine release, it attenuated the vancomycin-induced nephrotoxicity in rabbits. Therefore, it is unlikely that the attenuating effects of the antimicrobial agents on vancomycin-induced nephrotoxicity in rabbits is accounted for only by their effects on vancomycin-induced histamine release.

Fosfomycin sodium stabilizes lysosomes and mast cell membranes. When rat mast cells were incubated with compound 48/80, which was known to induce a rapid and strong degranulation with histamine release, the percentage of degranulated cells was 82%, but after pre-incubation with fosfomycin sodium, the degranulation percentage was 60% (Greco et al 1978). During heart-lung preparation of guinea-pigs, 1 mg fosfomycin sodium inhibited histamine release induced by aminoglycosides (Bertelli & Giovannini 1980). Furthermore, pre-incubation of rat mast cells significantly with fosfomycin sodium reduced the decrease in cAMP induced by compound 48/80, while it prevented the effect of 48/80 on cGMP (Schinetti et al 1979). It was also shown that 100 μg mL^{-1} or 1000 μg mL^{-1} fosfomycin sodium suppressed not only IgE-mediated histamine release but also histamine release by the Ca ionophore A 23187 or the synthetic peptide, formyl-methionyl-leucyl-phenylalanine (FMLP) in human peripheral blood leucocytes (Ida et al 1987).

In the present study, we investigated the histamine release ranging over the mole ratios of the vancomycin daily dose in humans to the other antimicrobial agents' daily doses. It was suggested that fosfomycin sodium might inhibit the vancomycin-induced histamine release. This favourable action might be added to the antibacterial synergic action of fosfomycin sodium and vancomycin (Takahashi et al 1987), and the protection of vancomycin-induced nephrotoxicity. Fluconazole, however, was suggested to provoke histamine release less than the miconazole level. Therefore, based on these findings, either fosfomycin sodium

or fluconazole may be suitable as a second antimicrobial agent to combine with vancomycin therapy.

References

Bertelli, A., Giovannini, L. (1980) Fosfomycin inhibition of pulmonary histamine release induced by aminoglycosides. Drugs Exp. Clin. Res. 6: 329–332

Gietzen, K., Penka, L., Eisenburger, R. (1996) Imidazole fungicides provoke histamine release from mast cells and induce airway contraction. Exp. Toxic. Pathol. 48: 529–531

Greco, R., Schinetti, M. L., Bertelli, A. (1978) Phosphomycin activity on rat mast cells. Proceedings of the 1st Mediterranean Congress of Chemotherapy, Madrid, 741–750

Heel, R. C., Brogden, R. N., Pakes, G. E., Speight, T. M., Avery, G. S. (1980) Miconazole: a preliminary review of its therapeutic efficacy in systemic fungal infections. Drugs 19: 7–30

Horinouchi, Y., Abe, K., Kubo, K., Oka, M. (1993) Mechanisms of vancomycin-induced histamine release from rat peritoneal mast cells. Agents Actions 40: 28–36

Ida, S., Shindoh, Y., Takishima, T. (1987) Effect of antibiotics on immediate hypersensitivity reactions in vitro: suppression of IgE-mediated histamine release from peripheral blood basophils by fosfomycin. Microbiol. Immunol. 31: 975–984

Levy, J. H., Kettlekamp, N., Goertz, P., Hermens, J., Hirshman, C. A. (1987) Histamine release by vancomycin: a mechanism for hypotension in man. Anesthesiology 67: 122–125

Polk, R. E. (1991) Anaphylactoid reactions to glycopeptide antibiotics. J. Antimicrob. Chemother. 27 (Suppl. B): 17–29

Renz, C. L., Laroche, D., Thurn, J. D., Finn, H. A., Lynch, J. P., Thisted, R., Moss, J. (1998) Tryptase levels are not increased during vancomycin-induced anaphylactoid reactions. Anesthesiology 89: 620–625

Sahai, J., Healy, D. P., Garris, R., Berry, A., Polk, R. E. (1989) Influence of antihistamine pretreatment on vancomycin-induced red-man syndrome. J. Infect. Dis. 160: 876–881

Schinetti, M. L., Greco, R., Giovannini, L., Bertelli, A. (1979) In vitro effects of phosphomycin on intracellular cyclic nucleotides and histamine releases. Drugs Exp. Clin. Res. 5: 363–365

Sullivan, T. J., Parker, K. L., Stenson, W., Parker, C. W. (1975) Modulation of cyclic AMP in purified rat mast cells. J. Immunol. 114: 1473–1479

Takahashi, K., Kanno, H., Chen, R. M. (1987) Effects of combination of vancomycin, fosfomycin, minocycline and cefamandole against Methicilline-resistant staphylococcus aureus. Chemotherapy 39: 180–183

Toyoguchi, T., Nakagawa, Y. (1996) Nephrotoxicity and drug interaction of vancomycin (2). Folia Pharmacol. Jpn 107: 225–235

Toyoguchi, T., Takahashi, S., Hosoya, J., Nakagawa, Y., Watanabe, H. (1997) Nephrotoxicity of vancomycin and drug interaction study with cilastatin in rabbits. Antimicrob. Agents Chemother. 41: 1985–1990

Toyoguchi, T., Takahashi, S., Hosoya, J., Nakagawa, Y. (1998) Nephrotoxicity and drug interaction of vancomycin in rabbits (4). Jpn. J. Hosp. Pharm. 24: 1–7

Tsuruta, Y., Kohashi, K., Ohkura, Y. (1978) Determination of histamine in plasma by high-speed liquid chromatography. J. Chromatogr. 146: 490–493

Wallace, M. R., Mascola, J. R., Oldfield, E. C. (1991) Red man syndrome: incidence, etiology, and prophylaxis. J. Infect. Dis. 164: 1180–1185

Williams, P. D., Laska, D. A., Shetler, T. J., McGrath, J. P., White, S. L., Hoover, D. M. (1991) Vancomycin-induced release of histamine from rat peritoneal mast cells and a rat basophil cell line (RBL-1). Agents Actions 32: 217–223