Potentiation of Vancomycin-Induced Histamine Release by Muscle Relaxants and Morphine in Rats

HIDEKI SHUTO, MASANORI SUEYASU, SHUJI OTSUKI, TOMOKO HARA, YUKI TSURUTA, YASUFUMI KATAOKA, AND RYOZO OISHI*

Department of Hospital Pharmacy, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan

Received 2 October 1998/Returned for modification 26 March 1999/Accepted 19 September 1999

The intravenous injection of vancomycin sometimes causes anaphylactoid reactions, in which histamine release may play a major role. These reactions are more frequently manifested when vancomycin is injected into anesthetized patients. We examined the vancomycin-induced histamine release and the interaction of vancomycin with muscle relaxants or opioid in rats. In an in vitro study with rat peritoneal mast cells, treatment with vancomycin at concentrations of greater than 1.25 mM produced significant histamine release. Tubocurarine, vecuronium, pancuronium, succinylcholine, and morphine up to concentrations of 0.25, 1, 5, 30, and 5 mM, respectively, produced no significant histamine release. However, the nonsignificant histamine release induced by 0.5 mM vancomycin was clearly enhanced by combining vancomycin with any of these agents. In the in vivo study, the intravenous injection of vancomycin significantly increased the plasma histamine levels in rats when vancomycin was injected at 200 mg/kg of body weight (63.2 ± 34.0 ng/ml [mean ± standard deviation]) but not when it was injected at 100 mg/kg (30.8 ± 20.2 ng/ml), compared with that in the saline-treated rats (22.5 ± 11.4 ng/ml). Although the subcutaneous administration of morphine (10 mg/kg) never increased the plasma histamine levels, the intravenous injection of vancomycin (100 mg/kg) 30 min after this morphine treatment markedly increased the plasma histamine levels (56.0 ± 26.9 ng/ml). These findings provide experimental evidence that the combination of muscle relaxants or an opioid with vancomycin may increase the risk of anaphylactoid reactions by enhancing the release of histamine.

Vancomycin is a tricyclic glycopeptide antibiotic obtained from cultures of Streptomyces orientalis. It is particularly useful in the treatment of serious infections caused by gram-positive bacteria resistant to β-lactam antibiotics. The most common untoward effects of intravenously administered vancomycin are anaphylactoid reactions characterized by pruritis, erythema, flushing, and, so on; the extreme flushing is sometimes called “red-man (neck) syndrome” (1, 9, 14). The infusion rate is believed to be the most important risk factor for red-man syndrome. It has been suggested that these anaphylactoid reactions in patients treated with vancomycin result from a vancomycin-evoked histamine release from mast cells and/or basophils (10, 13, 16, 25).

When vancomycin is injected into anesthetized patients, the anaphylactoid reactions mentioned above are more frequently manifested (18, 19, 23, 24). Vancomycin administration should therefore be avoided in patients under anesthesia. The manufacturer of vancomycin discourages its administration to anesthetized patients and recommends, instead, a slow infusion before the induction of anesthesia. However, there is no experimental basis for this policy. In the perioperative period, many drugs including general and local anesthetics, some opioids, plasma expanders, and muscle relaxants are administered to patients. In patients under anesthesia, anaphylactoid reactions commonly occur in response to these drugs (15, 16). It is also known that tubocurarine, vecuronium, pancuronium, and succinylcholine (which are all muscle relaxants) (4, 5, 7) and morphine (20, 21) have weak stimulatory effects on the release of histamine. However, the effects of the interaction between these drugs and vancomycin on the release of histamine remain unclear. We therefore conducted in vitro and in vivo studies to test whether muscle relaxants and morphine enhance the vancomycin-induced histamine release from mast cells and/or basophils.

Materials and Methods

Animals. Male Sprague-Dawley rats (Kyushu University Institute of Laboratory Animals, Etochu, Japan) weighing 300 to 350 g were housed in an air-conditioned room at 23 ± 2°C with free access to food and water and were maintained on a 12-h light, 12-h dark schedule (lights on at 7:00 a.m.). This experiment was reviewed by the Committee on the Ethics of Animal Experiments in the Faculty of Medicine, Kyushu University, and was carried out under the control of the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and The Law (No. 105) and Notification (No. 6) of the government of Japan.

Drugs. Vancomycin hydrochloride (purity, approximately 100%) and pancuronium bromide were purchased from Sigma Chemical Co. (St. Louis, Mo.). Morphine hydrochloride was from Sankyo Co. (Tokyo, Japan). Vecuronium bromide was kindly donated by Sankyo Co. All other chemicals were of reagent grade.

Histamine assay. The histamine levels were determined by ion-pair high-performance liquid chromatography (HPLC) coupled with postcolumn fluorometric derivatization as described previously (11). The HPLC system consisted of two multipumps (CCPM-II; Tosoh, Tokyo, Japan), an autosampler (AS-8020-LV; Tosoh), a guard column (μBondapak C18 Guard Pak; Millipore Co., Bedford, Mass.), a reversed-phase separation column (150 by 4.6 mm [inside diameter]) packed with TSK gel ODS-80TM (Tosoh), a thermostat reactor (RE-8020; Tosoh), and a fluorescence spectrometer (RF-550; Shimadzu, Kyoto, Japan). The mobile phase was 0.16 M KH₂PO₄ containing 0.1 mM sodium octanesulfonate (pH 4.5), and the flow rate was 0.6 ml/min. In this assay, the amount of histamine injected was linearly related to the fluorescence intensity from 0.5 to 1.000 pg, and the detection limit was 0.5 pg. None of the drugs used in the present studies interfered with the fluorometric assay.

Histamine release from rat peritoneal mast cells. The rats were anesthetized with ether and were exsanguinated by cutting one of the carotid arteries. Then, 20 ml of ice-cold Hanks’ balanced salt solution (HBSS; which consisted of NaCl, 137 mM; KCl, 3.56 mM; MgSO₄, 0.20 mM; Na₂HPO₄, 0.34 mM; KH₂PO₄, 0.44 mM; NaHCO₃, 4.17 mM; CaCl₂, 1.26 mM; glucose, 5.6 mM) was injected into the peritoneal cavity. After gentle massage of the abdomen for 90 s, the intra-peritoneal fluid was collected with a plastic pipette. Usually, the cells collected from two to four rats were pooled and used for one set of experiments. Further purification of mast cells was not performed. Various concentrations of drugs...
dissolved in HBSS (except for vancomycin, which was dissolved in water) were prepared. Each aliquot of the mast cell suspension (10^4 cells) in HBSS was preincubated at 37°C for 5 min. Each agent (0.1 ml) alone or in combination with vancomycin (0.1 ml) was added to the cell suspension (0.9 or 0.8 ml, respectively) and the mixture was incubated for another 5 min at 37°C. The reaction was terminated by placing the test tubes in crushed ice. After centrifugation (4°C, 125 × g, 10 min), the upper 500 μl of the supernatant was carefully transferred to another plastic tube, and 10 μl of 5.1 N perchloric acid was added. To the lower fraction, 500 μl of HBSS and 20 μl of 5.1 N perchloric acid were added, and the mixture was ultrasonicated. Each mixture was centrifuged (15,000 × g, 5 min), and the 20-μl aliquots of the supernatant were injected into the HPLC system. The extent of the histamine release (histamine in the supernatant) from the mast cells is expressed as the percentage of the total histamine content (intracellular plus supernatant histamine).

Change in plasma histamine levels. In the experiment for detection of an interaction between morphine and vancomycin, morphine was subcutaneously administered at doses of 5 and 10 mg/kg of body weight 30 min before vancomycin (100 and 200 mg/kg intravenously) administration. Rats were lightly anesthetized with ether 3 min after the vancomycin administration. The abdominal cavity was opened and blood samples were collected by puncture of the abdominal aorta for the measurement of plasma histamine levels. After the collection of blood, the plasma was immediately separated by centrifugation (4°C, 2,000 × g, 10 min) and was stored at −70°C until analysis. To 500 μl of plasma sample, 10 μl of 5.1 N perchloric acid was added, and the mixture was then vortexed for 10 min and centrifuged (15,000 × g, 5 min). Twenty-microliter aliquots of the supernatant were injected into the HPLC system.

Statistical analysis. Results are given as the means ± standard deviations (SDs). The significance of differences was calculated by one-way analysis of variance with post hoc Scheffe’s F test for individual comparisons. Differences were regarded as significant if P was <0.05.

RESULTS

Histamine release induced by vancomycin, muscle relaxants, and morphine from rat peritoneal mast cells. Over a concentration range of from 1.25 to 10 mM vancomycin induced the release of histamine from nonpurified rat peritoneal mast cells in a concentration-dependent manner (data not shown). The nonspecifically released (base) histamine content was 27.4 ± 5.1 ng/10^4 cells during a 5-min period and was 8.0% ± 1.5% of the total histamine content in the cells (340.9 ± 44.5 ng/10^4 cells). The histamine releases induced by vancomycin at 1.25 and 10 mM reached 21.7% ± 3.8% and 92.0% ± 2.5%, respectively. Figure 1 shows the dose-response curves of the histamine releases induced by muscle relaxants and morphine.

The minimal effective concentrations of tubocurarine, vecuronium, pancuronium, succinylcholine, and morphine required to stimulate a significant histamine release compared with the amount released nonspecifically were 0.5, 1.5, 7.5, 50, and 7.5 mM, respectively.

Effects of muscle relaxants and morphine on vancomycin-induced histamine release from rat peritoneal mast cells. The effects of tubocurarine, vecuronium, pancuronium, and succinylcholine at concentrations below their minimal effective concentrations on the histamine release induced by vancomycin were examined. Tubocurarine (0.1 mM), vecuronium (1 mM), pancuronium (5 mM), and succinylcholine (20 mM) markedly enhanced the histamine release induced by a low concentration (0.5 mM) of vancomycin compared with that induced by vancomycin alone (Fig. 2). Morphine at 1 and 3 mM, which are concentrations below the minimal effective concentration, facilitated the histamine release evoked by vancomycin at con-
centrations ranging from 0.5 to 2 mM in a dose-dependent manner (Fig. 3).

**Interaction between vancomycin and morphine on plasma histamine levels in rats.** Figure 4 shows the effect of morphine on the vancomycin-induced histamine release in vivo. The histamine level in rat plasma was 22.5 ± 9.6 ng/ml. Vancomycin significantly increased the plasma histamine levels when it was injected at 200 mg/kg (63.2 ± 34.0 ng/ml) but not when it was injected at 100 mg/kg (30.8 ± 30.2 ng/ml). The subcutaneous administration of morphine (5 and 10 mg/kg) never increased the plasma histamine levels. However, the intravenous injection of 100 mg of vancomycin per kg 30 min after treatment with 10 mg of morphine per kg significantly increased the histamine levels (56.0 ± 26.9 ng/ml). The increased histamine levels induced by 200 mg of vancomycin per kg were further enhanced by the pretreatment with 10 mg of morphine per kg, although there was no significant difference.

**DISCUSSION**

The results of the present study demonstrated that muscle relaxants and morphine enhance the vancomycin-induced histamine release in vitro and/or in vivo. These findings provide experimental evidence supporting several clinical reports that vancomycin administration to anesthetized patients increases the risk of anaphylactoid reactions.

Since there has been no study on the histamine release induced by muscle relaxants, morphine, and vancomycin under the same conditions in vitro, we first investigated the ability of each drug to induce the release of histamine from rat peritoneal mast cells. Vancomycin induced the histamine release over a concentration range from 1.25 to 10 mM, with a maximum release of 92%. This concentration range was almost identical to that reported by Horinouchi et al. (10), but the maximum release in this study was much higher than the maximum release that they reported (66%). They used rat peritoneal mast cells purified with Ficoll, while we used cells that were not purified. The purification of mast cells can induce the loss of the membrane component and can produce notable changes in the response (2, 6). The difference in the maximum release may thus be due to the purification. Among the agents tested, tubocurarine was the most powerful histamine-releasing agent. The effects of pancuronium, vecuronium, succinylcholine, and morphine on histamine release were much less than that of vancomycin. Vecuronium induced significant histamine release at a concentration lower than those required for induction of histamine release by morphine and pancuronium. Pancuronium induced the same histamine release as morphine did. In the in vitro experimental system used, a considerably higher concentration of morphine was required to induce a significant histamine release. However, the results are in good agreement with those reported previously (8).

Second, we examined the interactions between vancomycin and muscle relaxants or morphine in vitro. The vancomycin-
induced release of histamine from rat peritoneal mast cells was significantly enhanced by the addition of each of these agents. In addition, even at the concentration at which each agent alone had no effect on histamine release, the combination with vancomycin (e.g., 0.5 mM vancomycin and 1 mM morphine) resulted in a significant histamine release. We also examined the changes in plasma histamine levels to confirm whether the interaction between vancomycin and morphine observed in vitro is reproducible in vivo. The intravenous injection of vancomycin 30 min after the subcutaneous administration of morphine markedly increased the plasma histamine levels in the treated rats compared with those in the saline-treated rats. Thus, we clarified in vitro and in vivo that the combination of morphine with vancomycin significantly increases the amount of histamine released. This finding is the first evidence suggesting a synergistic histamine-releasing interaction between vancomycin and morphine. It has been suggested that vancomycin directly opens Ca\textsuperscript{2+} channels to induce histamine release (10). On the other hand, morphine is thought to directly activate G proteins (3). The rise in intracellular Ca\textsuperscript{2+} levels induced by these different mechanisms might have contributed to this synergistic action on histamine release from mast cells.

It has been reported that both the area under the concentration-time curve and the peak concentrations of histamine in serum show a significant correlation to the severity of erythema induced by vancomycin (22). Levy et al. (13) and Lyton and Bruce (17) also showed that the hypothensive effect of vancomycin infusion results exclusively from the histamine release and not from the direct myocardial depression. Many drugs are used during the perioperative period, including general and local anesthetics, some opioids, plasma expanders, and muscle relaxants. Among these drugs, muscle relaxants and opioids have weak stimulatory effects on histamine release. Anesthetics are not known to promote histamine release, and in fact, it has been reported that the tubocurarine-induced histamine release is inhibited by halothane (12). It can thus be argued that the anaphylactoid reactions produced by vancomycin in the perioperative period are not derived from the interaction with anesthetics or stress. In light of the present findings, the possibility that muscle relaxants or opioids enhance the vancomycin-induced histamine release should be considered in the manifestation of anaphylactoid reactions by vancomycin injection under anesthesia.

Vancomycin has been used more frequently in recent years as methicillin-resistant staphylococcus strains have been increasing. Opioids are widely used as analgesics at high doses to supplement the general anesthesia for various surgical procedures. In these clinical situations, the histamine release might occur more frequently during the perioperative period. A histamine release during anesthesia or surgery can cause serious consequences, such as hypotension, tachycardia, and bronchospasms. Clinicians should therefore carefully monitor the signs and symptoms of patients with anaphylactoid reactions and consider the benefits of routine antihistamine prophylaxis.

REFERENCES


