Enhancement by Morphine of Radiographic Contrast Media-Induced Histamine Release in Rat Peritoneal Mast Cells

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Received February 19, 1997 Accepted April 7, 1997

ABSTRACT—The intravascular application of radiographic contrast media causes hypersensitivity reactions, in which histamine release may play a major role. We examined the interaction between contrast medium and morphine. Among the four nonionic contrast media examined, iopamidol showed the most marked histamine release from rat peritoneal mast cells in vitro. Although iopamidol and morphine themselves did not induce histamine release at concentrations up to 65 mg/l/ml and 3 mM, respectively, their combination resulted in a significant histamine release. These findings suggest that patients with exposure to medicines that induce histamine release may have a higher incidence and severity of hypersensitivity reactions to radiographic contrast media.

Keywords: Radiographic contrast media, Morphine, Histamine release

Iodinated radiographic contrast media (RCM) are widely used for image enhancement in several diagnostic procedures. However, many undesirable adverse reactions to RCM including allergic, renal, vascular and cardiac effects have been reported (1). Even the nonionic RCM with lower osmolarity induce these reactions, although the incidence has been reported to be reduced as compared with ionic RCM (2).

Non-immunologic anaphylactoid reactions are also commonly associated with RCM infusion procedures (1). Anaphylactoid reactions are unpredictable and dose-independent and may manifest as bronchospasm, airway edema, gastrointestinal distress, urticaria and angioedema. The pathogenic mechanism of anaphylactoid reactions observed during RCM infusion is still unclear. The results of in vitro and in vivo studies suggest that histamine release from basophils and/or mast cells plays a major role in these reactions (3, 4). Several pretreatment regimens including $H_1$- and $H_2$-antagonists for the prophylaxis of anaphylactoid reactions to RCM have been studied (5).

Some risk factors for anaphylactoid reactions have been reported such as personal history of allergy/atopy, cardiovascular diseases and previous reactions to RCM (6, 7). However, there has been no report regarding the interaction with other medicines possessing the histamine-releasing effect. If RCM are administered to patients taking such medicines, the incidence and severity of anaphylactoid reactions might increase. In this study, we examined the interaction between RCM and morphine in the histamine release from rat peritoneal mast cells, because morphine is widely used, especially for the relief of cancer pain, and is also known to have histamine releasing action (8, 9).

Male Sprague-Dawley rats (Kyushu University Institute of Laboratory Animals), weighing 300–350 g, were anesthetized with ether and exsanguinated by cutting one of the carotid arteries. Then, 20 ml of ice-cold Hanks' balanced salt solution (HBSS; 137 mM NaCl, 5.36 mM KCl, 0.20 mM MgSO4, 0.34 mM Na2HPO4, 0.44 mM KH2PO4, 4.17 mM NaHCO3, 1.26 mM CaCl2 and 5.6 mM glucose) was injected into the peritoneal cavity. After gentle massage of the abdomen for 90 sec, the intraperitoneal fluid was collected with a plastic pipette. Usually the cells collected from 2–4 rats were pooled and used for one set of experiments. The mast cells were separated from the peritoneal fluid by centrifugation at 125 × g for 5 min at 4°C, washed twice with HBSS and then finally resuspended in HBSS.

Various concentrations of RCM diluted with distilled water and morphine dissolved in HBSS were prepared. Each aliquot of the mast cells (1 × 10⁴) suspension in HBSS was preincubated at 37°C for 5 min. RCM (1.0 ml) alone or in combination with various concentrations of...
morphine (0.1 ml) were added to the cell suspensions (0.5 and 0.4 ml, respectively) and incubated for another 5 min at 37°C. The reaction was terminated by placing the test tubes in crushed ice. After centrifugation (125 × g, 5 min, 4°C), the upper 0.75 ml of the supernatant was carefully transferred to another plastic tube, and 15 μl of 5.1 N perchloric acid was added. To the lower fraction, 0.75 ml of HBSS and 30 μl of 5.1 N perchloric acid were added, and the mixture was then ultrasonicated. The histamine contents of both fractions were determined with ion-pair HPLC coupled with postcolumn fluorescent derivatization (10). None of the drugs used in the present study interfered with the fluorometric assay. 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 + supernatant histamine).

The nonionic RCM used were iohexol (Omnipaque 300; Daiichi Pharmaceutical Industries, Tokyo), iomeprol (Iomeron 300; Eizai, Tokyo), iopamidol (Iopamiron 300; Nihon Schering, Tokyo) and ioversol (Optiray 320; Yamanouchi Pharmaceutical Industries, Tokyo). The concentration of RCM is expressed as iodine concentration (mg/ml) which, in radio-opacity, is the critical parameter ensuring a clear picture. Morphine hydrochloride was obtained from Sankyo Co. (Tokyo). Other chemicals were of reagent grade.

Data are expressed as means±S.E.M. (n=4). Significance of the difference was calculated by analysis of variance with post-hoc Scheffe's F-test for individual comparison by statistical analysis software (StatView; Abacus Concepts, Inc., Berkeley, CA, USA). Differences were regarded as significant at P < 0.05.

First, we examined the histamine releasing effects of four nonionic RCM in rat peritoneal mast cells. The spontaneous histamine release determined in HBSS without RCM was 5.7±0.5% (Fig. 1). Among the RCM examined, iopamidol showed the greatest histamine release at 135 mg/ml (20.3±0.5%). Iomeprol and ioversol also showed significant histamine release at the same concentration (9.2±0.2 and 10.5±0.7%, respectively). At 200 mg/ml, these three RCM showed significant histamine releases, but to extents almost the same as those at 135 mg/ml. The iohexol-induced histamine release was not significantly different from spontaneous histamine release, although the extent was similar to those by iomeprol and ioversol.

Figure 2 shows the interaction between morphine and iopamidol (the most potent histamine releaser of the four RCM in the first experiment) in the histamine release from rat peritoneal mast cells. Morphine itself did not induce histamine release at concentrations up to 3 mM, but synergistically enhanced histamine release induced by iopamidol over the concentration range tested. The non-significant histamine release induced by iopamidol at 20 mg/ml (9.7±0.4%) was clearly increased to 15.6±1.3%, 47.7±2.4% and 81.4±2.4% by the combination with 1 mM, 3 mM and 10 mM of morphine, respectively.

Since hyperosmolarity may be responsible for some of the adverse reactions caused by RCM (11, 12), new RCM with lower osmolarity have been developed. With the use of these novel preparations, the incidence of overall adverse reactions was reduced from 12.66% of patients who received higher osmolar RCM to 3.31%, and the incidence of severe anaphylactoid reactions (sudden drop in

![Fig. 1. Histamine release induced by radiographic contrast media from rat peritoneal mast cells. Each column represents the mean±S.E.M. of 4 experiments. RCM concentrations: 20 mg/ml, 65 mg/ml, 135 mg/ml, 200 mg/ml. *P < 0.05, **P < 0.01 and ***P < 0.001, as compared with the spontaneous release (HBSS).]
blood pressure, cardiac arrest, loss of consciousness, or dyspnea requiring treatment) was reduced from 0.22% to 0.04% (2).

Similar to the incidence of anaphylactoid reactions, histamine releases induced by lower osmolar RCM have been reported to be lower than those induced by hyperosmolar RCM (13). In the present study, we used nonionic RCM with lower osmolarity. Histamine releases from rat peritoneal mast cells ranged from 9.2% to 20.3% at the concentration of 135 mgI/ml. These values are similar to those reported previously (13). Some risk factors for anaphylactoid reactions to RCM have been found (6, 7). As for the medicines, Lang et al. (14) reported that patients receiving beta-blockers had a significantly higher risk of anaphylactoid reactions, probably due to reduced beta-adrenergic responsiveness.

The present results are the first evidence of the positive interaction of histamine release by RCM with other medicines in vitro. Iopamidol-induced histamine release was synergistically enhanced by the addition of morphine. Even at concentrations at which iopamidol and morphine themselves induce no histamine release, the combination (e.g., 65 mgI/ml of iopamidol and 1 mM of morphine) resulted in significant histamine release. Non-specific histamine release from mast cells by various therapeutic drugs in high doses may occur by a rise in intracellular Ca$^{2+}$, but the detailed mechanism is still unknown (15). The positive interaction of histamine release by RCM with morphine might have been due to a decrease in the threshold for a rise in intracellular Ca$^{2+}$.

In the experimental system used, a considerably high concentration of morphine was required to induce histamine release. However, this is in good agreement with the previous report (8), and the histamine releasing action of morphine was observed in patients (9). These findings suggest that patients with exposure to medicines that induce histamine release may have a higher incidence and greater severity of anaphylactoid reactions to RCM. Great caution should be taken in the use of RCM in patients receiving such medicines.

**Acknowledgments**

This study was supported by Grants-in-Aid for Science Research from the Ministry of Education, Science, Sports and Culture of Japan (No. 08672615).

**REFERENCES**

5 King J, Rothenberger KH and Claus W: Prevention of anaphylactoid reactions after radiographic contrast media infusion by combined histamine H$_{1}$- and H$_{2}$-receptor antagonists: results of a prospective controlled trial. Int Arch Allergy Appl Immunol 78, 9–14 (1985)
8 Ellis HV III, Johnson AR and Moran NC: Selective release of histamine from rat mast cells by several drugs. J Pharmacol Exp Ther 175, 627–631 (1970)
13 Akagi M and Tasaka K: Comparative study of the adverse effects of various radiographic contrast media, including iover-
