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Short communication

Nonimmunological release of histamine from rat mast cells elicited by antineoplastic agents: effect of drug combinations*

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Summary. We studied the release of histamine elicited by some antineoplastic drugs in rat pleural and peritoneal mast cells. The drugs tested included the antibiotic mitomycin; the anthracyclines daunorubicin, doxorubicin, and epirubicin; the glycopeptide bleomycin; the chromopeptide dactinomycin; the platinum coordination complexes carboplatin and cisplatin; and the anthracenedione mitoxantrone. Mitomycin and bleomycin failed to elicit any release of histamine, whereas all of the other drugs induced histamine release from either pleural or peritoneal mast cells, the release being independent of extracellular calcium. The results indicate that antineoplastic drugs activate histamine release in a manner similar to that shown by compound 48/80, which may contribute to some of their secondary effects.

Introduction

Hundreds of drugs have been described as histamine releasers on mast cells from several species [9]. The reason why so many different agents activate exocytosis on mast cells is unknown, since no structure-activity relationship exists among them. The main characteristic common to all of these drugs is their basic nature (e.g., compound 48/80, substance P, D-tubocurarine, and morphine) [6]. The mechanism by which these drugs activate the mast cells is not known, but the signal transduction pathways to these stimuli include the activation of different kinases through G-proteins [8] and the enhancement of cytosolic Ca^{2+} concentrations [10]. The direct activation of guanidine triphosphate (GTP)-binding proteins seems to be necessary for the action of all of these stimuli [1, 11].

The therapeutic use of antineoplastic drugs has been accompanied on some occasions by the appearance of side effects due to the nonimmunological release of histamine by, i.e., cisplatin [17] or anthracyclines [16]. We have systematically studied the action that most of the antineoplastic agents currently in use exert on rat mast cells. Some of these results have been reported elsewhere [4]. In this report we present new evidence that some antineoplastic drugs elicit histamine release via direct activation of mast cells. Since rat pleural and peritoneal mast cells are two pharmacologically different populations [2], we carried out separate studies on these cell populations.

Materials and methods

Chemicals. Since antineoplastic drugs do not follow any structure-activity relationship, we used the classification system of the European Pharmaceutical Marketing Association, which classifies drugs according to their mechanism of action or chemical structure. This paper reports the results we obtained using the following classes of agents: (a) *antibiotics*, including the chromomycin mitomycin; the anthracyclines daunorubicin, doxorubicin, and epirubicin; the glycopeptide bleomycin; and the chromopeptide dactinomycin; and (b) *miscellaneous*, including the platinum coordination complexes carboplatin and cisplatin and the anthracenedione mitoxantrone. Mitomycin was obtained from Inibsa (Spain); daunorubicin, doxorubicin, and epirubicin were supplied by Farmitalia (Italy); bleomycin was obtained from Almirall (Italy); and dactinomycin was supplied by Merck (FRG). Orthophthalaldehyde was obtained from Merck (FRG), and all other chemicals were supplied by Sigma (USA).

Mast-cell isolation. Mast cells were obtained by lavage of pleural and peritoneal cavities of Sprague-Dawley rats (200–400 g) as previously described [3]. The physiological saline comprised 142.3 mM Na^+, 5.94 mM K^+, 1 mM Ca^{2+}, 1.2 mM Mg^{2+}, 126.1 mM Cl^-, 22.85 mM CD_3^-, 1.2 mM $PO_4\ H_2^-$, and 1.2 mM SO_4^{2-}, giving a final osmotic pressure of 300± 5 mosmol/kg H_2O. Bovine serum albumin (1 mg/ml) was added and the pH was adjusted to 7.0. The unpurified cellular suspension contained 4%–8% mast cells, with the average being $1.5-2\times10^6$ mast cells/rat. Cell viability was determined using the trypan blue exclusion test [2] and was always higher than 97%.

Cell incubation. In all, 25 µl of a freshly prepared, concentrated solution of each drug was added to sufficient incubation medium to attain a final volume of 0.9 ml, and the mixture was preincubated. When the medium had reached 37° C, 100 µl cell suspension containing $1-1.5\times10^5$ mast cells was added to each tube. Incubations were carried out in a bath at

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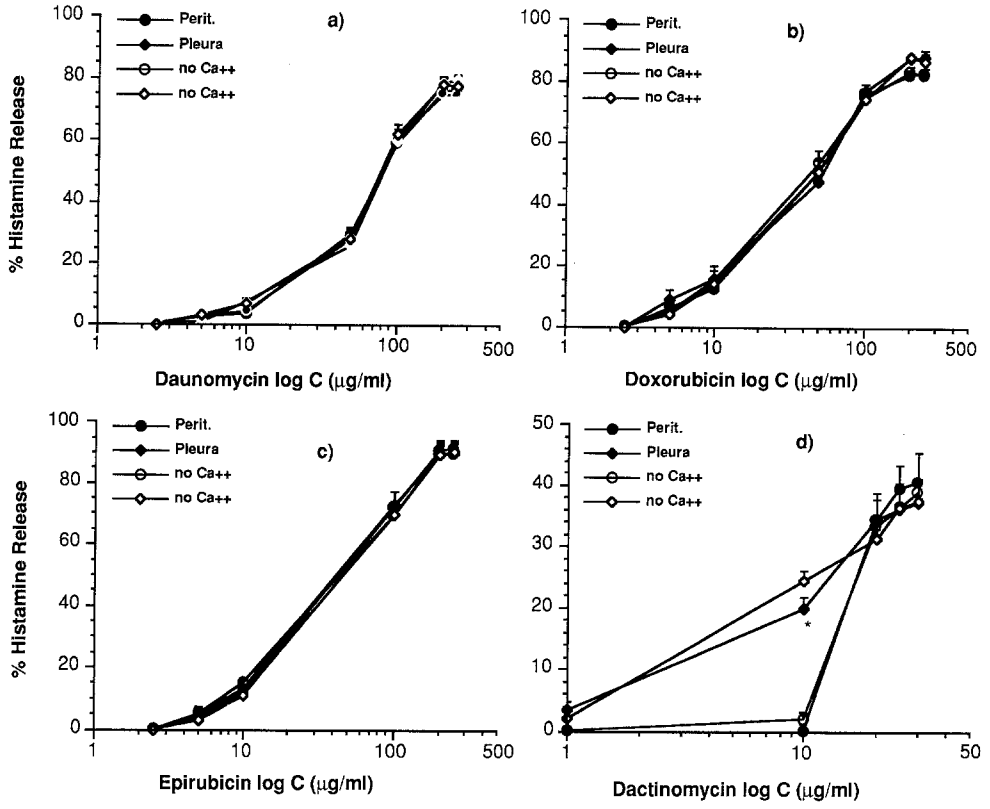

Fig. 1 a–d. Histamine release from rat pleural (*Pleura*) and peritoneal (*Perit.*) mast cells stimulated with different concentrations of **a** daunorubicin, **b** doxorubicin, **c** epirubicin, and **d** dactinomycin in the presence and absence of extracellular calcium. Significant differences with respect to controls are indicated with an asterisk. Data represent mean values ± SEM for 4 experiments

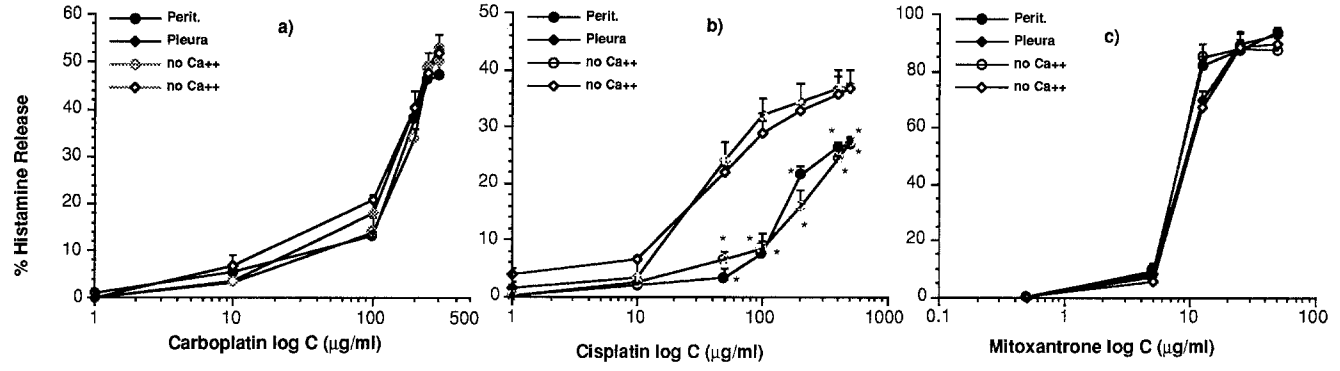

Fig. 2 a–c. Histamine release from rat pleural (*Pleura*) and peritoneal (*Perit.*) mast cells stimulated with different concentrations of **a** carboplatin, **b** cisplatin, and **c** mitoxantrone in the presence and absence of extracellular calcium. Data represent mean values ± SEM for 4 experiments

37° C for 10 min, and samples were incubated for an additional 10 min following the addition of the stimulus. Incubations were stopped by immersion of the tubes in a cold bath. After centrifugation at a maximal velocity of 1000 *g* for 5 min, the supernatants were collected and decanted into other tubes for histamine determination. Appropriate control determinations of spontaneous histamine release in the absence of stimuli were executed during every experiment.

Histamine-release assay. Histamine was assayed spectrofluorometrically both in the pellet (residual histamine) and in supernatants (released histamine) according to Shore's method [15], the exception being that 0.1% orthophthalaldehyde was used. Trichloroacetic acid was added (final concentration, 7%), to prevent reactions because protein interferes with histamine assay. To establish the total histamine content, pellets were sonicated for 60 s in 0.8 ml 0.1 *N* ClH. For each drug, we studied the possible release of histamine caused by each excipient alone or combined as in the commercial preparation. The controls showed that no histamine release was elicited by the excipients. Moreover, the osmotic pressure generated by the combination of drugs plus excipients failed to induce any release of histamine. The results were expressed as the percentage of histamine released with respect to the total histamine content.

Statistical analysis. Results were analyzed using Student's *t*-test for unpaired data. A probability value of $P < 0.05$ was considered to represent statistical significance. All results were expressed as mean values ± SEM.

Results

Mitomycin and bleomycin failed to elicit any release of histamine in either pleural or peritoneal mast cells of rats. Figure 1 a shows the response of pleural and peritoneal mast cells to daunorubicin. Both populations showed the same pattern of response in the presence or absence of extracellular calcium. Maximal histamine-release values

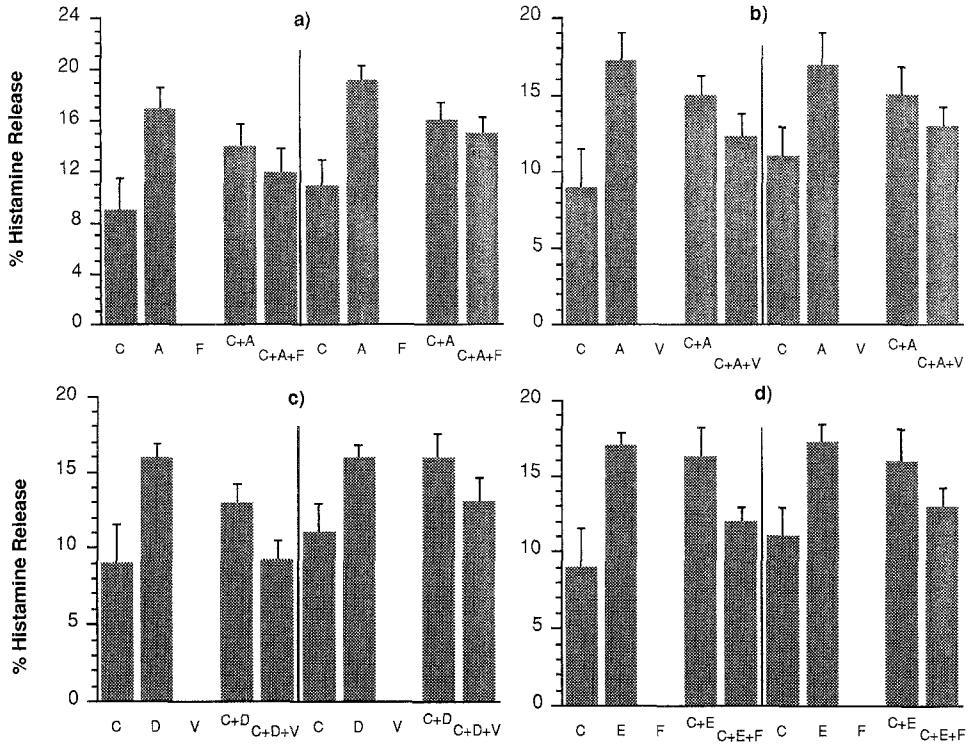

Fig. 3 a–d. Histamine release from rat pleural and peritoneal mast cells stimulated with different combinations of antineoplastic drugs. Each plot is divided by a *vertical line*, with data obtained in peritoneal cells appearing on the *left*. The combinations are explained in detail in Results

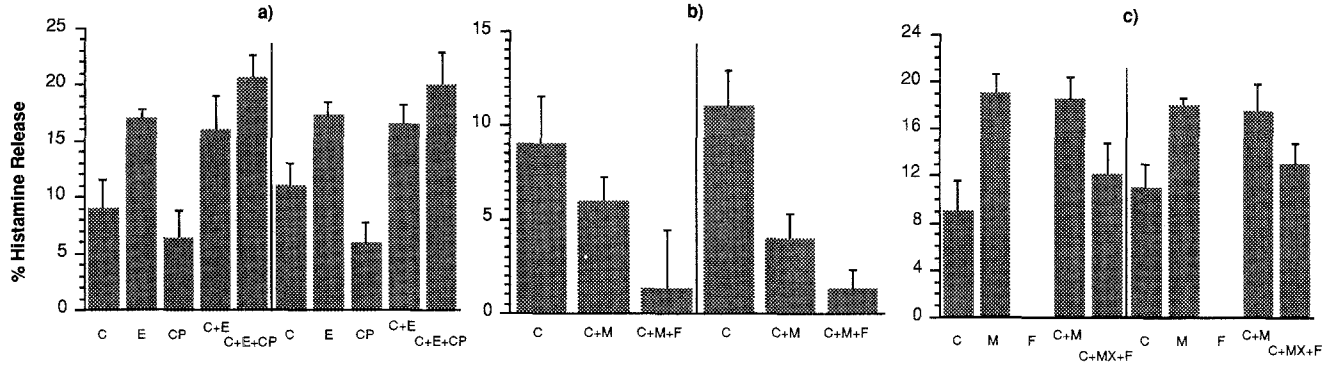

Fig. 4 a–c. Histamine release from rat pleural and peritoneal mast cells stimulated with different combinations of antineoplastic drugs. Each plot is divided by a *vertical line*, with data obtained in peritoneal cells appearing on the *left*. The combinations are explained in detail in Results

reached approximately 80%, and the cells started to release histamine at a daunorubicin concentration of 10 μg/ml. The same type of response is shown in Fig. 1 b for doxorubicin; maximal release values reached 85%, and the cells started to release histamine at a drug concentration of 5 μg/ml. Again, a similar profile of response was obtained using epirubicin; maximal histamine-release values for pleural and peritoneal mast cells reached 90%, and the response was triggered by an epirubicin concentration of 5 μg/ml (Fig. 1 c). Figure 1 d shows the response of these cell populations to dactinomycin. Pleural mast cells were 10 times more sensitive to this agent than were peritoneal cells, the triggering concentration being 1 and 10 μg/ml for pleural and peritoneal cells, respectively. Maximal histamine-release values reached approximately 40% in both populations following incubation with dactinomycin at 30 μg/ml. The absence of extracellular calcium did not affect the response of either of the two populations.

Figure 2 a shows the response of mast cells to carboplatin. Maximal histamine-release values reached 50%, and the triggering concentration was 10 μg/ml. The response profile was not affected by the absence of extracellular calcium. Figure 2 b illustrates the response to cisplastin. In this case, pleural mast cells displayed a much greater sensitivity to the drug, the maximal response also being higher. The absence of calcium did not change the profile of the response of either pleural or peritoneal cells. The maximal release values reached 25% in peritoneal mast cells and 38% in pleural mast cells. Figure 2 c shows a nondiscriminative response by pleural and peritoneal mast cells in both the presence and the absence of calcium following their stimulation with mitoxantrone. Histamine release was elicited by a drug concentration of 5 μg/ml, and maximal release values approached 90% under all conditions tested.

Figures 3 and 4 show the release of histamine by mast cells in the presence of calcium following their treatment with drug combinations commonly used in clinical therapy. We chose some of the many possible combinations, and each plot shows the histamine-release values obtained using the drugs as single agents and as combination regimens. To detect possible interactions and summative responses, we used 20% of the usual dose of all drugs except those that have been reported not to induce histamine release, in which case we used a higher concentration (100 μg/ml). The drugs we used in these combinations that belong to the latter group included 5-fluorouracil, vincristine, and methotrexate [4]. The combinations that we tested were cyclophosphamide (C)-doxorubicin (A)-5-fluorouracil (Fig. 3a), cyclophosphamide-doxorubicin-vincristine (V); (Fig. 3b), cyclophosphamide-daunorubicin-vincristine (Fig. 3c), cyclophosphamide-epirubicin(E)-5-fluorouracil (Fig. 3d), cyclophosphamide-epirubicin-cisplatin (CP); (Fig. 4a), cyclophosphamide-methotrexate (M)-5-fluorouracil (Fig. 4b), and cyclophosphamide-mitoxantrone (MX)-5-fluorouracil (Fig. 4c). In none of these combinations did we find summative effect; on the contrary, in situations in which two drugs were acting simultaneously, the total response was smaller than the sum of the responses of the respective controls.

Discussion

The main observation arising from the present results is that antineoplastic drugs that induce histamine release do so absolutely independently of extracellular calcium. The drugs described in this report as new histamine releasers exhibit no structure-activity relationship; therefore, the activation of mast cells by these agents is not mediated by specific receptors and probably takes place through G-proteins as in the case of basic releasers [12]. This can be explained if the mechanism of activation of the cells resembles that of compound 48/80. The histamine release elicited by anthracyclines has previously been studied [5, 14], and our results essentially support those previous findings.

The differing sensitivity of pleural and peritoneal mast cells to dactinomycin and cisplatin confirms the heterogeneity of these two populations as well as previous data showing that the pleural population is generally more reactive [3, 7]. The histamine-releasing activity of all of these drugs may also be operative in human mast cells and could therefore explain some secondary effects of the use of these agents, i.e., rat mast cells are pharmacologically very similar to human skin mast cells, and the therapeutic use of asparaginase is accompanied by a high incidence (65%) of allergic skin reactions in cases in which a reaction is detected [13, 17]. We would not attempt to attribute the histamine release elicited by all these drugs to mechanistic effects, but we find it interesting that pleural and peritoneal cells showed different responses to dactinomycin and cisplatin. A similar disparity in response has previously been reported for etoposide and cytarabine [4]. In contrast to the responses previously obtained using ifosfamide or cytarabine [4], the response to all of the drugs tested in the present study remained stable in the absence of extracellular calcium.

One striking result, which is difficult to explain, involved the lack of summative effects following treatment of the cells with drug combinations. There are several possible explanations for this phenomenon: (a) the drugs may interact with the cell in the same places and therefore might simply compete for the same binding site (however, this possibility does not explain the lack of summative effects between drugs such as cyclophosphamide and doxorubicin or cyclophosphamide and epirubicin) or (b) the drugs may interact in different places in the cell, activating antagonistic actions that result in a lower release. Although we do not propose any mechanistic explanation for this finding, investigations of this aspect could improve our understanding of the nature of the cellular interaction of antineoplastic drugs.

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