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Short communication

Non-immunological release of histamine from rat mast cells elicited by antineoplastic agents*

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Summary. We studied the histamine-releasing activity of several antineoplastic drugs on rat pleural and peritoneal mast cells. The drugs tested included the nitrogen mustards cyclophosphamide and ifosfamide, the nitrosourea carmustine, the triazene dacarbazine, the folic acid analogue methotrexate, the pyrimidine analogue cytarabine and fluorouracil, the vinca alkaloids vinblastine, vincristine and Vinorelbine, the epipodophyllotoxins etoposide and teniposide, and the enzyme L- asparaginase. Methotrexate, carmustine, fluorouracil, vinblastine and vincristine failed to elicit histamine release on rat mast cells. All of the other drugs evoked histamine release in both the presence and the absence of extracellular calcium, but ifosfamide, cytarabine and asparaginase induced a much lower release in the absence of this cation. The response elicited by cytarabine and etoposide was much higher in pleural than in peritoneal mast cells. These results indicate that some antineoplastic drugs may directly activate the release of histamine, which could contribute to some of their secondary effects.

Introduction

Hypersensitivity reactions to antineoplastic drugs can be mediated by immunological or non-immunological stimulation of mast cells. Reactions to some drugs such as L-asparaginase are often mediated by IgE, but several patients have reacted to their first drug dose [6], suggesting that L-asparaginase can also release vasoactive substances non-immunologically. The same non-immunological activation of mast cells has been observed for cisplatin [15] and anthracyclines [8]; in fact, the non-immunological release of histamine by anthracyclines may explain their cardiotoxicity [5].

Rat mast cells represent a cellular model that is widely used for the study of exocytosis. An important characteristic of these cells is the pharmacological heterogeneity that they exhibit, depending on the anatomical origin and the species [2]. Although rat serosal mast cells are pharmacologically similar to human-skin mast cells, the results obtained in one population cannot be definitively applied to the other [7]. Taking into account these limitations, we undertook an in vitro study of most of the antineoplastic drugs that are currently used in clinical therapy to determine both whether or not they induce the release of histamine on rat mast cells in vitro and the influence of extracellular calcium on the response they evoke. The study was carried out independently on pleural and peritoneal mast cells, since these two populations are pharmacologically different [3]. In the present paper we report as histamine releasers drugs that have not previously been described as such.

Materials and methods

Chemicals. Since antineoplastic drugs do not exhibit any structure-activity relationship, we applied the classification system of the European Pharmaceutical Marketing Association, which classifies drugs according to their mechanism of action or chemical structure. In the present paper we report the results obtained using the following groups of compounds: (a) alkylating agents – the nitrogen mustards cyclophosphamide and ifosfamide, the nitrosourea carmustine and the triazene dacarbazine; (b) antimetabolites – the folic acid analogue methotrexate and the pyrimidine analogue cytarabine and fluorouracil; and (c) natural products – the vinca alkaloids vinblastine, vincristine and Vinorelbine, the epipodophyllotoxins etoposide and teniposide, and the enzyme L-asparaginase. Cyclophosphamide and ifosfamide were obtained from Funk (FRG); carmustine, etoposide and teniposide, from Bristol-Myers (USA); methotrexate, from Almirall (Italy); dacarbazine, from Farmitalia (Italy); cytarabine, from Upjohn (England); fluorouracil, from Roche (Switzerland); vinblastine and vincristine, from Lilly (USA); Vinorelbine, from Pierre Fabre (France); and L-asparaginase, from Bayer (FRG). All other chemicals were supplied by Sigma (USA), and Orthophthalaldehyde was obtained from Merck (FRG).

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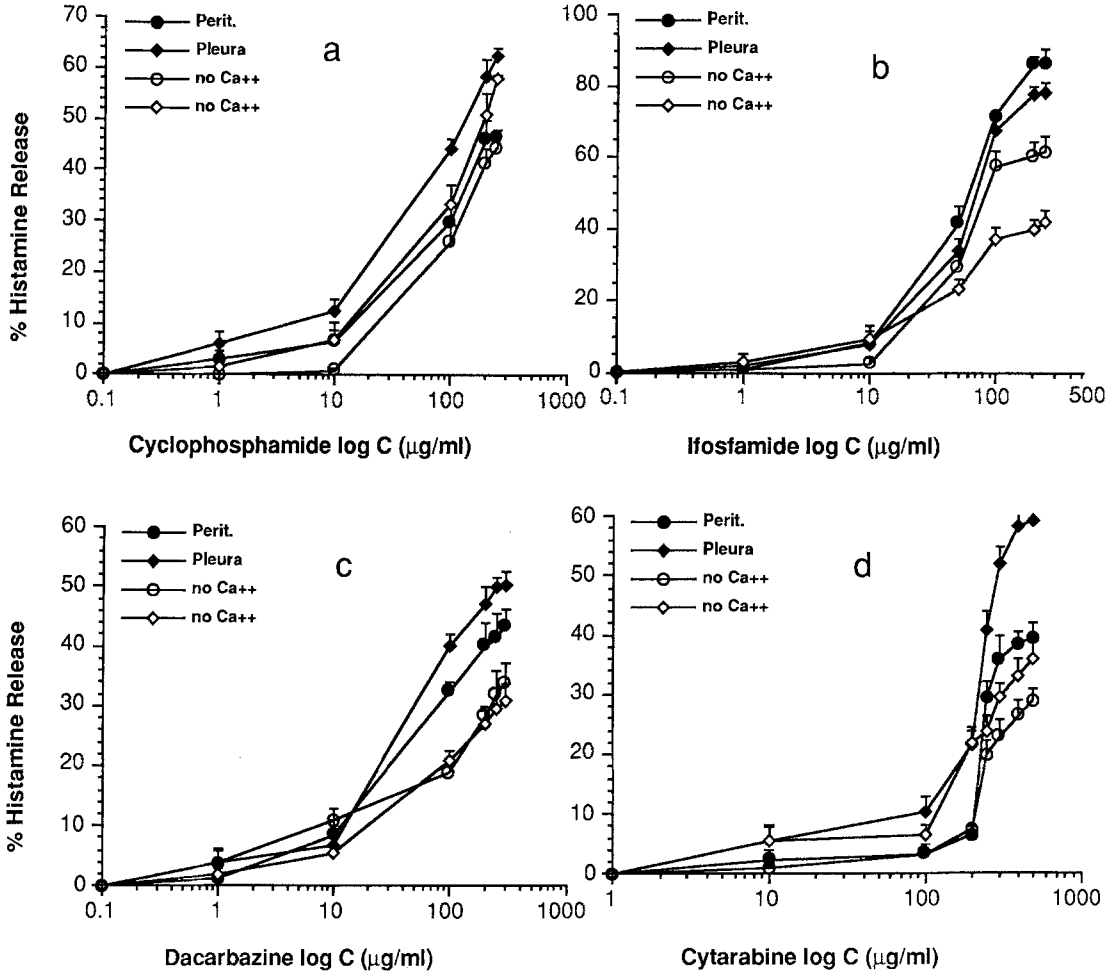

Fig. 1 a–d. Histamine release elicited by **a** cyclophosphamide, **b** ifosfamide, **c** dacarbazine and **d** cytarabine on rat pleural (*Pleura*) and peritoneal (*Perit.*) mast cells in the presence and absence of extracellular calcium (mean values ± SEM for 4 experiments). The results obtained in the absence of extracellular calcium are statistically significantly different from the control values at the following points of the curves: **a** pleura point 4; **b** pleura, points 5–7 and peritoneum, points 6, 7; **c** pleura and peritoneum, points 4–7; **d** pleura, points 5–8 and peritoneum, points 6–8. Point 1 corresponds to the initial concentration

Mast-cell isolation. Mast cells were obtained by lavage of the pleural and peritoneal cavities of Sprague-Dawley rats (200–800 g) as previously described [12]. The composition of the physiological saline was Na^+, 142.3 mM; K^+, 5.94 mM; Ca^{2+}, 1 mM; Mg^{2+}, 1.2 mM; Cl^-, 126.1 mM; CO_3^-, 22.85 mM; $PO_4H_2^-$, 1.2 mM; SO_4^-, 1.2 mM, 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. The mast-cell content of the unpurified cellular suspension was 4%–8%, for an average of $1.5–2 \times 10^6$ mast cells/rat. Cell viability was studied by the trypan-blue exclusion test [3] and was always >97%.

Cell incubation. In all, 25 ml 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 reached 37° C, 100 μl cell suspension containing $1–1.5 \times 10^5$ mast cells was added to each tube. Incubations were carried out in a water bath at 37° C for 10 min and then for 10 min additionally after the addition of the stimulus. Incubations were stopped by immersion of the tubes in a cold bath. After centrifugation at a maximum of 1000 *g* for 5 min, the supernatants were collected and decanted into other tubes for histamine determination. Appropriate controls for the determination of spontaneous histamine release in the absence of stimuli were prepared in every experiment.

Histamine-release assay. Histamine was assayed spectrofluorometrically in both the pellet (residual histamine) and the supernatants (released histamine) using Shore's method [14] except that 0.1% Orthophthalaldehyde was employed. Trichloroacetic acid was added (final concentration, 7%) to prevent reactions, as protein interferes with histamine assay. To ensure that all histamine was obtained, pellets were sonicated for 60 s in 0.8 ml 0.1 *N* HCl. For each drug, we studied the possible release of histamine caused by each excipient either alone or combined as in the commercial preparation. In the controls, no histamine release was elicited by the excipients; in addition, the osmotic pressure generated by combination of drugs plus excipients failed to induce any release of histamine. The results were expressed as a percentage of the histamine released with respect to the total histamine content.

Statistical analysis. The results were analyzed using Student's *t*-test for unpaired data, whereby a probability level of ≤ 0.05 was considered to represent statistical significance. All results were expressed as mean values ± SEM.

Results

Methotrexate, carmustine, fluorouracil, vinblastine and vincristine failed to elicit histamine released on rat mast cells. Figure 1 a shows the release of histamine on mast cells that were stimulated with cyclophosphamide. Pleural mast cells showed a higher response than peritoneal mast

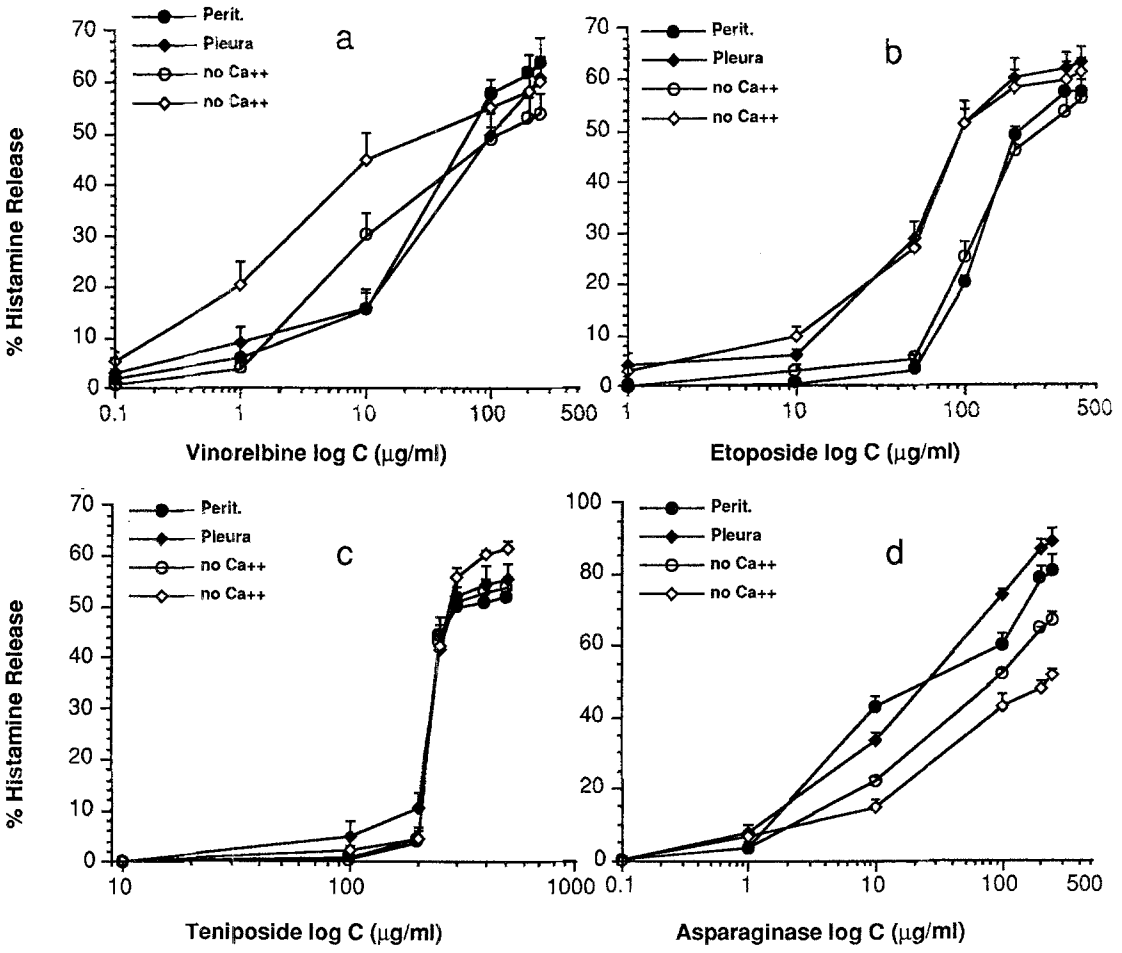

Fig. 2 a–d. Histamine released elicited by **a** Vinorelbine, **b** etoposide, **c** teniposide and **d** asparaginase on rat pleural (*Pleura*) and peritoneal (*Perit.*) mast cells in the presence and absence of extracellular calcium (mean values ± SEM for 4 experiments). The results obtained in the absence of extracellular calcium are statistically significantly different from the control values at the following points of the curves: **a** pleura, points 2, 3 and peritoneum, point 3; **d** pleura, points 3–6 and peritoneum, point 3. Point 1 corresponds to the initial concentration

cells. The absence of calcium did not significantly change the pattern of the response, with maximal histamine-release values of 44.5% ± 3.5% and 58% ± 1% being obtained for peritoneal and pleural mast cells, respectively. Stimulation of the cells with ifosfamide induced a different pattern of response in the absence of calcium (Fig. 1 b). Both cell populations were sensitive to the absence of extracellular calcium, with pleural cells being much more sensitive, showing a reduction of 50% in their response as compared with the control value whereas the maximal reduction for peritoneal cells was 25%.

Figure 1 c shows the response of mast cells to dacarbazine. Both pleural and peritoneal cells released approximately the same amount of histamine in the presence of calcium, and both populations showed similarly lower release in the absence of extracellular calcium. The lower release value was significantly different only with respect to the pleural control. Figure 1 d illustrates the release of histamine on cells that were stimulated with cytarabine. The response of pleural mast cells was considerably higher than that of peritoneal mast cells, with histamine-release values of 60% ± 4% and 40% ± 3%, respectively, being obtained. In the absence of extracellular calcium, histamine release was inhibited by 28% and 40% in peritoneal and pleural mast cells, respectively.

The response of pleural and peritoneal cells to Vinorelbine (Fig. 2 a) reached the same maximal values, but these two cell populations showed a clear difference in their sensitivity to the drug within the concentration values of 1–10 µg/ml. In the absence of extracellular calcium, the response of the cells was reduced, notably that of the pleural mast cells, in which a 66% inhibition was induced by treatment with 10 µg/ml. In contrast, treatment of the cells with a 66% inhibition was induced by treatment with 10 µ/ml. In contrast, treatment of the cells with etoposide (Fig. 2b) resulted in a clear difference in the responsiveness of the two populations. Pleural mast cells began releasing histamine following incubation with etoposide concentrations 10 times lower than those necessary to activate the peritoneal mast cells. The maximal response was similar in both cases, with histamine-release values of approximately 60% being recorded. It is noteworthy that extracellular calcium was not necessary for histamine release by the cells, since no inhibition was observed in its absence.

A quite different profile of response was obtained using teniposide (Fig. 2 c). No change occurred in the absence of extracellular calcium, and both populations showed the same sensitivity to each concentration of the drug in their release of histamine. As in the case of Vinorelbine, the

response to L-asparaginase (Fig. 2d) was elicited by relatively low concentrations of drug (1 μg/ml), and the maximal histamine-release value was about 85% for both pleural and peritoneal mast cells. Pleural mast cells were extremely sensitive to the lack of extracellular calcium, showing a reduction of 45% in their response.

Discussion

The present study shows that several of the chemotherapeutic drugs studied induce the release of histamine on both pleural and peritoneal mast cells. Mast cells are well known for their capacity to be activated by a great number of drugs that exhibit no common structure-activity relationship. Presumably, a common feature of the drugs that activate mast cells (i.e. compound 48/80, d-tubocurarine, morphine, substance P) is the basic nature of most but not all of them [10]. The mechanism by which these drugs activate the mast cells is not known, but the signal-transduction pathways to these stimuli involve the activation of different kinases through G-proteins [9] and increases in cytosolic Ca^{2+} concentrations [11]. The direct activation of guanosine triphosphate (GTP)-binding proteins seems to be a prerequisite for the action of all of these stimuli [1, 12].

The drugs described in this report as new histamine releasers exhibit no structure-activity relationship and therefore do not activate the cell through specific receptors. Therefore, we may assume that the mechanism of action involves that previously described as G-protein activation [1, 12]. Moreover, all of the agents tested induced more or less the same histamine-release pattern, which involved either no change or a slight inhibition of histamine release in the absence of extracellular calcium. Basically, this is the pattern of response previously found for the compound 48/80 [3].

One interesting observation was that pleural mast cells were generally more sensitive to the lack of extracellular calcium, and they usually released histamine at concentrations lower than those observed for peritoneal mast cells. This finding is in agreement with previous observations for other drugs [3, 4]. The histamine-releasing activity of all of these drugs may also be exerted on human mast cells and could therefore explain some of the secondary effects of the use of these agents; 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 [13] in cases in which a reaction is detected.

Although the aim of the present study did not include an explanation of the mechanism of action by which these antineoplastic drugs induce the release of histamine by mast cells, some points would be worthy of investigation, such as the question as to why pleural mast cells are so much more sensitive to etoposide or cytarabine than are peritoneal mast cells or why the lack of calcium causes such a considerable change in the response of pleural mast cells to ifosfamide or cytarabine.

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