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SHORT COMMUNICATION

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Adriamycin-induced histamine release from heart tissue in vitro

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Abstract It has been proven that the anthracyclines induce an important, noncytotoxic histamine release from rat peritoneal mast cells. As mast cells derived from different tissues exhibit marked heterogeneity, the effect of Adriamycin in comparison with other antineoplastic agents was tested on fragments of the right heart auricle, which contain a great number of mast cells. In this experimental model, Adriamycin induced a dose-dependent histamine release that was significantly limited by the antiexocytotic drug sodium cromoglycate. The antineoplastic agents cisplatin and 5-fluorouracil, in contrast, did not provoke any comparable histamine release. In the formulation employed in clinical settings, paclitaxel was also capable of inducing a histamine release comparable with that of Adriamycin; the exocytotic activity, however, was also evident when the tissue fragments were treated with Cremophor EL alone, without the addition of paclitaxel, whereas treatment of samples with paclitaxel dissolved in ethanol did not induce any releasing action. These data thus suggest that the secretory activity should be ascribed to the solvent Cremophor EL and not to paclitaxel. The release of histamine induced by paclitaxel in Cremophor EL/ethanol was also limited by sodium cromoglycate. These results again indicate that histamine release from mast cells derived not only from the peritoneal cavity but also from the cardiac tissue could play a role in the cardiotoxicity of anthracyclines and of paclitaxel in the clinically employed formulation.

Key words Adriamycin · Paclitaxel · Heart tissue · Histamine release · Cremophor EL

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Introduction

Cardiomyopathy is a unique characteristic of the anthracycline antibiotics. The pathogenesis of this side effect is not yet clear, and it has been suggested that it has multiple causes [18], but there is evidence that the release of histamine may be crucial in producing both acute and chronic cardiotoxicity. In previous studies we have shown that the anthracyclines induce an important histamine release from rat peritoneal mast cells in vitro [4], and that the mast-cell stabilizer sodium cromoglycate inhibits this exocytotic effect and significantly reduces the cardiac toxicity of Adriamycin and epirubicin [2, 5, 13].

Mast cells have been identified in heart tissues of animals [10] and humans [1, 7, 10, 23], and it has long been suspected that these cells play a pathophysiologic role in this tissue function [22]. Cardiac mast cells have also been implicated in various cardiomyopathies such as chronic chagasic cardiomyopathy [3], eosinophylic myocarditis [9], and idiopathic dilated cardiomyopathy [7, 11]. Histamine is present in the mammalian heart, and concentrations as high as 3 μg/g are found in the right atrium [17]. It has also been shown that various substances, including opioids, compound 48/80, calcium ionophore [8], local and general anesthetics, and contrast media [16] can induce the release of histamine from the heart tissue.

Mast cells derived from different anatomic sites display marked heterogeneity and differ in cell size, staining characteristics, ultrastructure, and, above all, mediator content and responsiveness to activating stimuli [19, 24, 29]. It therefore seemed of interest to study the pattern of responsiveness to the histamine-releasing action of Adriamycin in comparison with other antineoplastic agents using fragments of the right auricle of rat hearts. In addition, the effects of sodium cromoglycate were evaluated.

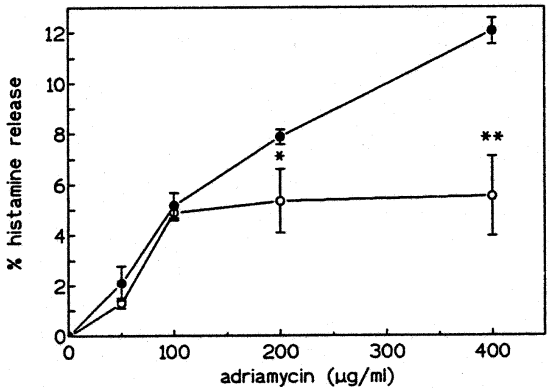

Fig. 1 Effect of sodium cromoglycate (2 m*M*) on Adriamycin-induced histamine release from rat heart. Fragments of right auricle were prewarmed for 5 min at 37 °C; Adriamycin and sodium cromoglycate were added and the incubation was continued at 37 °C for 45 min. Spontaneous histamine release (approx. 5%) was deducted (*Points* mean values [$n = 4-12$], *vertical bars* standard errors, ● Adriamycin alone, ○ Adriamycin + sodium cromoglycate). $*P < 0.05$; $**P < 0.01$ (significantly different from Adriamycin alone)

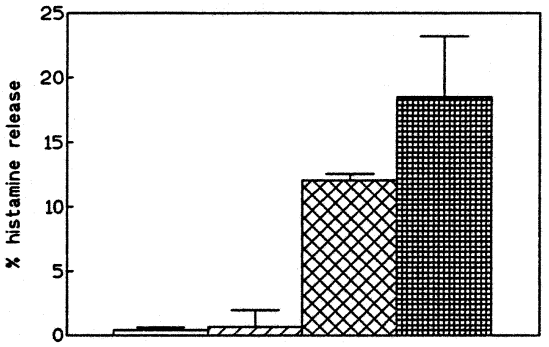

Fig. 2 Histamine release from rat heart induced by Adriamycin and other antineoplastic agents. Fragments of right auricle were prewarmed for 5 min at 37 °C; the antineoplastic drugs were added and the incubation was continued at 37 °C for 45 min. Spontaneous histamine release (approx. 5%) was deducted (*Columns* mean values [$n = 4-10$], *vertical bars* standard errors, □ 5-fluorouracil [1 mg/ml], ▨ cisplatin [500 µg/ml], ▩ Adriamycin [400 µg/ml], ▦ paclitaxel [250 µg/ml]). $*P < 0.05$; $**P < 0.01$ (significantly different from untreated controls)

Materials and methods

Study design

Wistar rats (200–400 g) belonging to a local conventional breeding colony were used. Animals were housed in groups of three at 20 °C on a 12-h light/dark cycle; all animals had free access to both food and water. Rats were anesthetised with ether and then killed by exsanguination. The heart was rapidly removed and flushed free of blood via a cannula inserted into the aorta. The right auricle was removed and tissue was chopped into approximately 1-mm^3 fragments and thoroughly washed in cold buffered saline solution (BSSA) to remove peripheral blood cells; BSSA had the following composition: NaCl 154 m*M*, KCl 2.7 m*M*, $CaCl_2$ 0.68 m*M*, Na_2HPO_4 10 m*M*, KH_2PO_4 10 m*M*, and bovine serum albumin 1 g/l, adjusted to pH 7.2. Tissue fragments were divided in individual tubes to obtain samples of approximately the same weight. Chopped tissue was incubated in quadruplicate in the presence of slowly bubbling O_2 for 45 min at 37 °C with various concentrations of the test substances in a metabolic shaker under gentle mechanical agitation.

At the end of the incubation period the samples were centrifuged at 150 *g* for 3 min at 4 °C, and 0.5 ml of 4% $HClO_4$ was added to 0.5 ml of the supernatants. The tissue fragments were sonicated in 1 ml of 2% $HClO_4$ to release residual histamine. All the samples were assayed for histamine using the ^{125}I-Histamin(e)-Ria Amicyl-Test (Immuno Biological Laboratories, Hamburg, Germany). As the acylation tubes work only at pH >7.4, a known quantity of phosphate buffer was added to all samples to obtain this pH value. Prior to the radioimmunoassay the sample preparation, i.e., derivatization of histamine to *n*-acylhistamine, was performed in active-ester-coated assay tubes. The derivatized histamine was subsequently measured using competitive binding with ^{125}I-conjugated tracer, and experimental values were read from a reference curve prepared using known standards.

The amout of histamine released was expressed as a percentage of the total histamine present in each sample. All values were corrected for spontaneous release occurring in drug-free samples (approximately 5%).

The release of the cytoplasmic enzyme lactate dehydrogenase (LDH) in the supernatants was measured as an indicator of cell viability (LDH/LD, Sigma Diagnostics). Averages ± SE of the mean values were calculated; statistical evaluation of the results was carried out using Student's *t*-test for independent samples. Values of $P < 0.05$ were considered significant.

Chemicals

Adriamycin, paclitaxel, 5-fluorouracil, cisplatin, sodium cromoglycate, and Cremophor EL were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were of analytical grade. All the test substances except paclitaxel were dissolved in BSSA. As the paclitaxel formulation for human administration consists of a solution of 6 mg drug/ml in a mixture of 50% (v/v) Cremophor EL and dehydrated alcohol, we dissolved and used paclitaxel from Sigma Chemical Co. in identical concentrations of Cremophor EL and ethanol, according to the instructions of the manufacturer, and paclitaxel dissolved only in ethanol. As a control we added Cremophor EL to the tissues at the same concentration used for investigation in combination with paclitaxel.

Results

The anthracycline antibiotic Adriamycin induces a dose-dependent histamine release from rat heart tissue in vitro that is limited by the mast-cell stabilizer sodium cromoglycate (Fig. 1). The antineoplastic agents cisplatin and 5-fluorouracil, tested at equitoxic doses, did not induce any comparable histamine release from rat cardiac auricles; in contrast, paclitaxel induced significant exocytosis, comparable with that of Adriamycin (Figs. 2, 3). It is noteworthy that histamine release was evident when paclitaxel was dissolved in Cremophor EL/ethanol, and almost identical results were obtained when mast cells were treated with Cremophor EL alone, without the addition of paclitaxel. In contrast, treatment with paclitaxel dissolved in ethanol did not induce any histamine-releasing action, suggesting that the secretory activity should be ascribed to the solvent Cremophor EL and not to paclitaxel. The cytotoxic effect of the tested antineoplastic drugs was evaluated by measurement of the release of LDH in the medium. No significant difference was evident between controls and treated samples (data not shown). Sodium cromoglycate also significantly reduced the histamine release induced by paclitaxel in Cremophor EL/ethanol (Fig. 3).

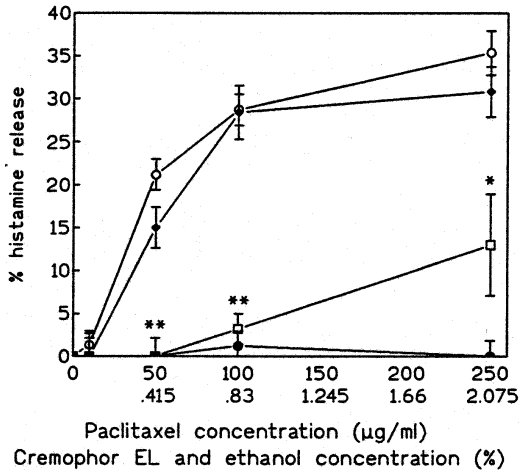

Fig. 3 Histamine release induced by paclitaxel dissolved in Cremophor EL/dehydrated ethanol (1/1, ◆), Cremophor EL alone at the same concentrations as when used together with paclitaxel (○), paclitaxel dissolved in Cremophor EL/ethanol plus sodium cromoglycate (2 m*M*; □), and paclitaxel dissolved in dehydrated ethanol (●) from rat heart. Fragments of right auricle were prewarmed for 5 min at 37 °C; the test substances were added and the incubation was continued at 37 °C for 45 min. Spontaneous histamine release (approx. 5%) was deducted. Each point represents the mean value ± SEM for 4–8 experiments. **P* <0.05; ***P* <0.01 (significantly different from paclitaxel in Cremophor EL/ethanol alone)

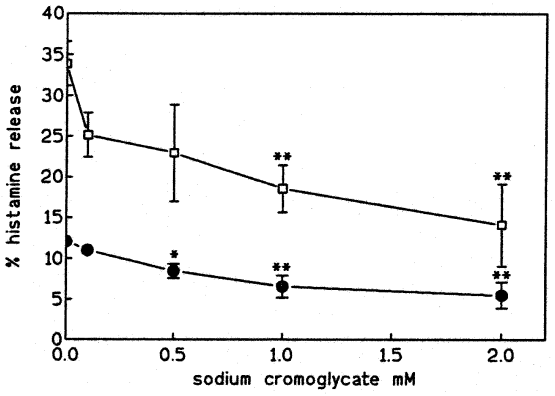

Fig. 4 Dose-dependent effect of increasing concentrations of sodium cromoglycate on the release of histamine induced by Adriamycin (400 µg/ml; ●) or by paclitaxel (250 µg/ml) dissolved in Cremophor EL/dehydrated ethanol (1/1; □) from rat heart. Fragments of right auricle were prewarmed for 5 min at 37 °C; the test substances were added and the incubation was continued at 37 °C for 45 min. Spontaneous histamine release (approx. 5%) was deducted. Each *point* represents the mean value ± SEM for 4 experiments. **P* <0.05; ***P* <0.01 (significantly different from paclitaxel in Cremophor EL/ethanol and Adriamycin alone)

The effect of increasing concentrations of sodium cromoglycate on the histamine-releasing action of Adriamycin and paclitaxel is depicted in Fig. 4; only the higher concentrations of the mast-cell stabilizer were effective.

Discussion

In previous studies [4] we have shown that the anthracyclines induce an important, noncytotoxic histamine release from rat peritoneal mast cells in vitro that is significantly inhibited by sodium cromoglycate. This exocytotic action has been correlated with the cardiotoxicity induced by these antineoplastic agents, as sodium cromoglycate almost completely protects animals from this side effect [2, 13].

Mast cells isolated from different anatomic sites, however, show marked heterogeneity in terms of their response to immunological and nonimmunological secretagogues [19, 24, 29]; hence, it seemed of particular interest to study the histamine-releasing action of Adriamycin in cardiac tissue, which is particularly rich in mast cells. Histamine has long been known to be present in the mammalian heart [1, 7, 10, 23], and mast cells have been implicated in various animal and human cardiomyopathies [3, 7, 9, 11].

In this report we show that Adriamycin also induces histamine release from fragments of rat auricles and that sodium cromoglycate can limit this release. As the heart tissue contains different cells, it is possible that Adriamycin might act directly on mast cells as well as on other cells, which, in turn, activate cardiac mast cells. The histamine-releasing action of Adriamycin is a true, noncytotoxic, exocytotic response; indeed, the release of LDH in the medium was not increased in treated samples as compared with controls. Sodium cromoglycate is effective in limiting Adriamycin- and paclitaxel-induced histamine release from rat auricles only at high concentrations; these data are in accordance with previous work from our laboratory [13] and other institutions [21] showing that the concentrations of the antiallergic drug that can reduce nonimmunological histamine release are at least 1 order of magnitude higher than those active on antigen-induced secretion. The inhibitory effect of sodium cromoglycate at 2 m*M* is maximal when Adriamycin is used at the two highest concentrations, whereas the compound has almost no effect at the two lowest concentrations. This phenomenon is difficult to explain, and it is conceivable that a small (<5%) amount of release cannot be completely counteracted. These data further stress the importance of histamine release in the pathogenesis of Adriamycin-induced cardiomyopathy and suggest that cromoglycate could be a pharmacological tool to prevent this side effect. The possibility that adriamycin might act on cells that, in turn, activate cardiac mast cells cannot be excluded; however, our previous data [4] indicating a true exocytotic effect of Adriamycin on isolated mast cells in vitro, together with the protective effect of sodium cromoglycate, suggest a direct effect of the antineoplastic drug on cardiac mast cells as well.

The exocytotic activity on myocardial tissue is peculiar to Adriamycin, as other antitumor agents tested at equitoxic doses, such as cisplatin and 5-fluorouracil, have not been shown to induce any comparable histamine release. Whereas to our knowledge, data have not appeared in the literature about a cardiotoxic effect for cisplatin, a number of studies have confirmed the cardiotoxicity of 5-fluorouracil in patients [14, 20]. Although the mechanism of 5-fluorouracil cardiotoxicity is not well understood, the stimulation of liberation of vasoactive substances has

been proposed as a contributing factor [14]. Our data, showing that this antineoplastic drug is not capable of inducing any histamine release from heart fragments in vitro, suggest that histamine does not have any role in this 5-fluorouracil-induced side effect. Accordingly, previous data from our laboratory have shown that this antineoplastic drug does not induce histamine release from rat peritoneal mast cells in vitro [4].

On the other hand, in the formulation employed for human administration, paclitaxel, an extremely potent antitumor agent [28], induces a significant histamine release comparable with that of Adriamycin. Interestingly, paclitaxel causes severe toxicity due to major hypersensitivity reactions, and histamine release by mast cells has been advocated as one possible mechanism [28]. Among the toxic side effects of paclitaxel is cardiotoxicity that is usually mild and reversible [25, 26], but severe cardiac complications have been reported, particularly in patients with preexisting heart disease [12, 25, 27]. The histamine-releasing activity was also evident when Cremophor EL alone is used without paclitaxel; in contrast, treatment with paclitaxel dissolved in ethanol did not induce any exocytotic action, suggesting that Cremophor EL is responsible for the release of histamine. These data are in accordance with previous observations conducted in rat peritoneal mast cells [6]. It is noteworthy that other drugs formulated in this polyoxyethylated castor oil, such as cyclosporine and vitamin K, have been associated with similar reactions [15].

In conclusion, there is evidence that Adriamycin and paclitaxel induce histamine release in heart tissues in vitro and that the antiallergic drug sodium cromoglycate limits the exocytotic activity of these substances. These observations further suggest an important role for histamine in the cardiac toxicity of these antineoplastic agents and a possible use of sodium cromoglycate in its prevention.

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