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Effect of Polyethylene Glycol 400 on Adriamycin Induced Histamine Release*

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Abstract—*The activity of polyethylene glycol* 400, *a widely used drug solvent, was tested on the release of histamine induced by adriamycin* in vitro *on peritoneal rat mast cells and* in vivo *in a mouse model.*

Preincubation of mast cells with high (10 *and* 5%) *concentrations of polyethylene glycol* 400 *significantly inhibited the important histamine release induced by* 100 μg/ml *of adriamycin; furthermore, polyethylene glycol* 400 (3.45 g/kg; 0.345 g/ml) *pretreatment almost completely abolished the peritoneal and pericardial mast cell degranulation and the cardiac toxicity caused by an intraperitoneal injection of* 15 mg/kg *of adriamycin.*

This effect of polyethylene glycol 400 *on adriamycin-induced histamine release could explain the protective action exhibited* in vivo *on adriamycin treated animals, therefore confirming that adriamycin cardiotoxicity could be related to the release of histamine and other vasoactive substances.*

INTRODUCTION

THE ANTHRACYCLINE antibiotics are extremely effective anticancer agents; however, the clinical use of these drugs has been limited by a dose-related cardiomyopathy [1]. The pathogenetic mechanism of adriamycin cardiotoxicity is not entirely understood and it probably has multiple causes. Recent works lead to the hypothesis that anthracycline cardiomyopathy is mediated by the release of vasoactive substances. In particular, in dogs [2] adriamycin produces acute cardiovascular effects that appear to be related to the release of histamine and catecholamines and to increased prostaglandin synthesis; in rabbits adriamycin induces a chronic cardiomyopathy which can be prevented by pretreatment with antihistamines and antiadrenergics [3]. Subacute adriamycin cardiotoxicity in rabbits can also be mediated by the release of cardiac histamine: pretreatment with disodium cromoglycate completely prevented histamine release from adriamycin in the isolated heart and produced significant protection against adriamycin induced subacute cardiomyopathy [4].

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In previous studies [5, 6] we have shown that pretreatment of mice with the widely used solvent polyethylene glycol 400 could prevent the cardiac toxicity induced by adriamycin. Among the several pharmacological and biological actions of polyethylene glycols some authors [7] have pointed out a reduction in spontaneous and polymyxin-B induced release of histamine, which increases respectively as the molecular weight of polyethylene glycols increases.

In the current study we examine the effects of a pretreatment with polyethylene glycol 400 on the release of histamine induced by adriamycin *in vitro* on mast cells isolated from the peritoneal cavity of rat and *in vivo* in a mouse model of cardiomyopathy.

MATERIALS AND METHODS

In vitro *studies*

Mixed peritoneal cells were obtained as previously described [8]; male Sprague–Dawley rats (200–400 g) were anesthetized with ether, and 15 ml of a buffered physiological solution warmed at 37°C were injected into their peritoneal cavity. The buffered solution consisted of 1.54×10^{-1} M NaCl, 2.7×10^{-3} M KCl, 9×10^{-4} M $CaCl_2$, 5.6×10^{-3} M D-glucose, human serum albumin 1 g/l and 10% by volume of a Sörensen buffer

containing 3×10^{-2} M $Na_2HPO_4 \times 7\ H_2O$ and 3.5×10^{-2} M $NaH_2PO_4 \times H_2O$. The pH of the solution was finally adjusted to 7 with 1 N NaOH. The abdomens were gently massaged for 1 or 2 min, the animals were decapitated and the fluid was removed by aspiration through an incision in the abdominal wall. The cells were sedimented by centrifugation at 200–250 × ***g*** for 10 min, the supernatant fraction was carefully removed, and the cells were resuspended in buffered medium. A pooled suspension from more rats was employed for a day's experiment (final cell suspension 2 ml of solution per rat). The cell suspension contained approximately 10% mast cells and was used without further purification since it has been shown that mast cells are the only source of histamine in the population of rat peritoneal cells [9, 10] consisting, in addition, of macrophage and mesothelial cells.

Aliquots (400 μl) of washed peritoneal cell suspension were preincubated at 37°C in a metabolic shaker with gentle mechanical agitation with the inhibitors for 30 min (200 μl of a doubly concentrated solution of the inhibitors in physiologic solution were added to 200 μl of the cell suspension), prior to exposure for 30 min to the histamine releasing agent (10 μl). After incubations were completed, tubes were centrifuged at about 200 × ***g*** for 3 min, and the supernatants were separated from cells. Histamine remaining in the cells was released by resuspending the cell pellets in 400 μl of the buffered solution, and allowing to stand in a boiling water bath for 10 min; the supernatants were treated similarly. The supernatants containing adriamycin could not be tested because of their own fluorescence; in these experiments only the residual histamine content of the mast cells was determined. One hundred μl aliquots of each fraction were assayed according to the fluorometric method of Shore *et al.* [11], omitting the extraction step. Preliminary investigations revealed that no extraction procedures were necessary to remove adriamycin since the limited quantity of the drug remaining in the cells accounted for a small (< 5%) and relatively constant fraction of the total fluorescence and hence did not significantly interfere with histamine measurements. The amount of histamine released was calculated as a percentage of the total histamine present in control suspension.

At the end of the incubation period, cell viability was estimated by trypan blue exclusion.

Average and S.E.M. were calculated; the probability that differences between means were chance occurrence was calculated using Student's *t*-test for independent samples.

In vivo *studies*

CD1 male mice with an average weight of 28–30 g were used. The animals were intraperitoneally treated with 15 mg/kg of adriamycin in NaCl 0.9%. A separate group of animals was pretreated with polyethylene glycol 400 (für die Gaschromatographie, E. Merck, Darmstadt) 3.45 g/kg (0.345 g/ml in distilled water) i.p., 3 hr before adriamycin. The mice were sacrificed 30 min, 1, 6 or 24 hr after adriamycin treatment; three animals per group were examined at the above-stipulated experimental time.

An autopsy was performed on all animals, and specimens of the liver, kidney, heart and of the pericardial and peritoneal membranes were collected and immediately frozen by immersion in liquid nitrogen or fixed in 8% buffered paraformaldehyde. The frozen material was cut using a Reichert mod 2700 cryostat and 12 μm thick sections were placed on slides and covered with Sörensen phosphate buffer saline : glycerin (9 : 1) at pH 8.5 and subsequently mounted in 60%

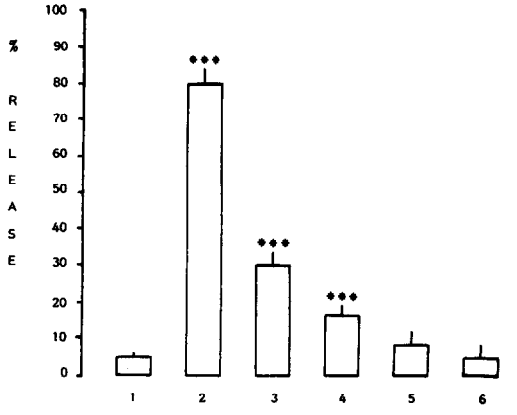


Fig. 1. Per cent release of histamine from peritoneal rat mast cells: 1. *controls*; 2. *adriamycin* 100 μg/ml; 3. *adriamycin* 50 μg/ml; 4. *adriamycin* 25 μg/ml; 5. *adriamycin* 12.5 μg/ml; 6. *adriamycin* 6.25 μg/ml. *Data are presented as means of* 4 *samples*; *vertical bars are S.E.M.* *** $P < 0.001$ *vs controls.*

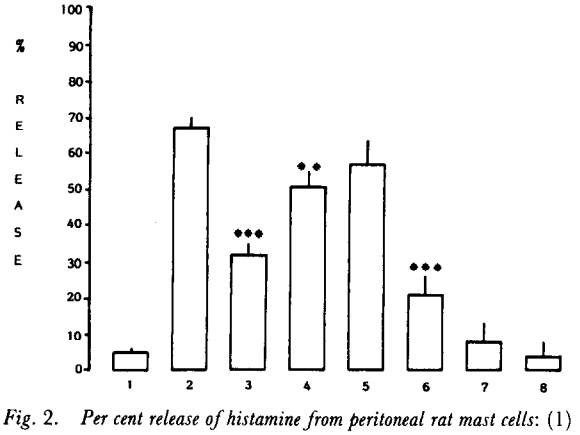
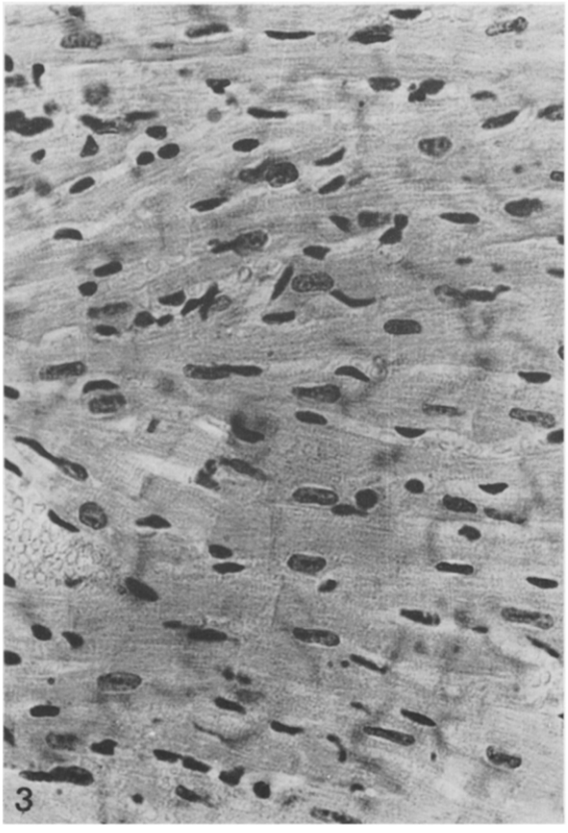
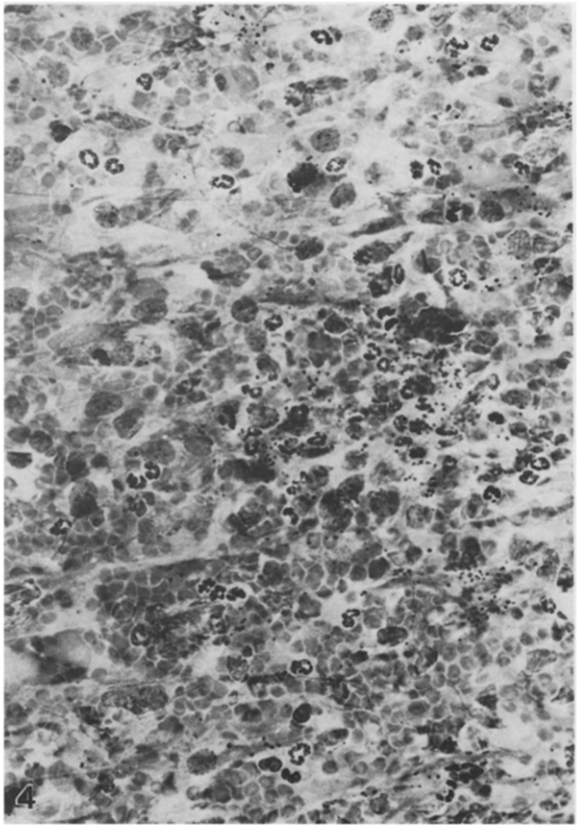
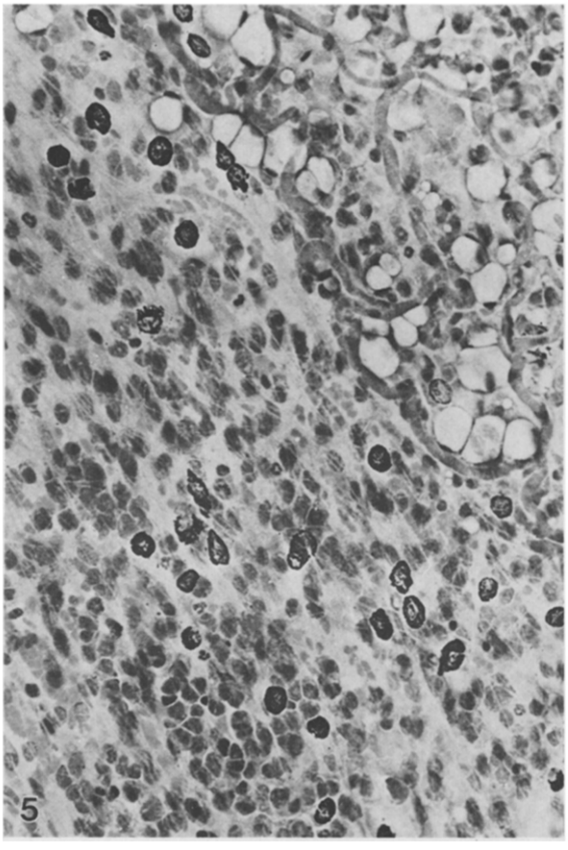


Fig. 2. Per cent release of histamine from peritoneal rat mast cells: (1) *controls*; (2) *adriamycin* 100 μg/ml; (3) *adriamycin* 100 μg/ml; + *polyethylene glycol* 400 10%; (4) *adriamycin* 100 μg/ml + *polyethylene glycol* 400 5%; (5) *adriamycin* 100 μg/ml + *polyethylene glycol* 400 0.5%; (6) *adriamycin* 100 μg/ml + *theophylline* 1.8 mg/ml; (7) *polyethylene glycol* 400 10%; (8) *theophylline* 1.8 mg/ml. *Data are presented as means of* 9 *samples* (10 *for controls*); *vertical bars are S.E.M.* ** $P < 0.01$ *vs adriamycin.* *** $P < 0.001$ *vs adriamycin.*







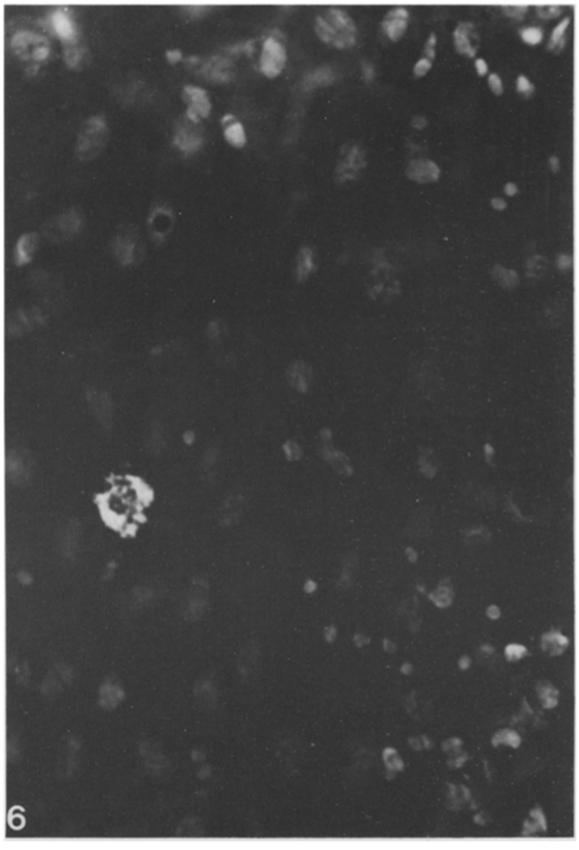


Fig. 3. *Light microscopic appearance of ventricular tissue from a mouse receiving adriamycin* 15 mg/kg *i.p.* + *polyethylene glycol* 400 3.45 g/kg *i.p. Toluidine blue* × 320.

Fig. 4. *Massive flogosis and mast cell degranulation in the visceral peritoneum from a mouse receiving adriamycin* 15 mg/kg *i.p. Toluidine blue* × 200.

Fig. 5. *Reduced flogosis and mast cell degranulation in the visceral peritoneum from a mouse receiving adriamycin* 15 mg/kg *i.p.* + *polyethylene glycol* 400 3.45 g/kg i.p. *Toluidine blue* × 200.

Fig. 6. *Visceral peritoneum from a mouse treated with adriamycin* 15 mg/kg *i.p.* + *polyethylene glycol* 400 3.45 g/kg *i.p.*; *under the fluorescence microscope adriamycin inside cell nuclei is fluorescent.* × 320.

aqueous glycerine [12]. These slides were examined in dark field with a Leitz Orthoplan high pressure lamp fluorescence microscope, using a BG12 excitation filter and K530 or K510 barrier filters. Photomicrographs were taken with an Agfachrome 50 L Professional colour film.

Some sections that had already been observed under the fluorescence microscope were stained with 1% aqueous solution of toluidine blue, mounted in 60% aqueous glycerine and then observed under the light microscope.

Paraformaldehyde fixed samples were embedded in paraffine and 7 μm thick sections were stained with hematoxilin and eosin and observed under the light microscope.

RESULTS

In vitro *studies*

Figure 1 shows that adriamycin induces a significant and dose dependent histamine release from rat mast cells at the three higher tested concentrations. Adriamycin induced histamine release is not related to the disruption of mast cells, as, after the considered incubation time, mast cells were still able to exclude trypan blue dye (viability : 98%).

Polyethylene glycol 400, at the two highest dosages, significantly limited the release of histamine induced by 100 μg/ml of adriamycin (Fig. 2); this concentration was chosen because it caused the most important release, without disruption of cells. The reduction produced by polyethylene glycol 400 is similar to that obtained using 1.8 mg/ml of theophylline, a classic inhibitor [13].

In vivo *studies*

The histological analysis of the organs of adriamycin treated animals showed the presence of the typical lesions induced by this drug; the early damage was particularly evident in the hearts and was almost completely prevented by pretreatment with polyethylene glycol 400 (Fig. 3). The observations on the peritoneal and pericardial membranes were of particular interest: adriamycin caused an important degranulation of the mast cells, which was more evident after 30 min, followed at 1, 6 and 24 hr, by a massive flogosis in the peritoneum and pericardium (Fig. 4). Pretreatment with polyethylene glycol 400 limited mast cell degranulation and subsequent inflammation (Fig. 5); however, under the fluorescence microscope, the mast cell nuclei appeared fluorescent, indicating that polyethylene glycol did not interfere with adriamycin entrance into the cells (Fig. 6).

DISCUSSION

The present study shows that treatment of mast cells with adriamycin provokes an important histamine release which can be partially prevented by a pretreatment with high concentrations of polyethylene glycol 400. These data are in agreement with *in vivo* observations: pretreatment of mice with polyethylene glycol 400 limited the peritoneal and pericardial mast cell degranulation and subsequent inflammation, and protects animals from adriamycin-induced acute and chronic [5, 6] toxicity.

Several recent works suggest that all types of anthracycline cardiac effects may be related to the release of histamine and other vasoactive substances. Cardiac histamine release has been documented in the isolated Langendorff preparations; this release appears to be specific for adriamycin, and could not be provoked by actinomycin D and compound 48/80 [3]; however, adriamycin administration also induced a significant increase in plasma levels of histamine [2, 14]. In addition, in the open chest dog [2], the acute cardiovascular effects of adriamycin appear to be completely related to the release of histamine and catecholamines and to increased prostaglandin synthesis. In rabbits [3], chronic cardiac effects can be prevented by pretreatment with antihistamines and antiadrenergics, and in the same animal model [4], cromolyn sodium, a compound that inhibits the secretion of histamine by mast cells stimulated by a variety of secretagogues [15], blocks cardiac histamine release and prevents the occurrence of subacute anthracycline cardiotoxicity in the majority of pretreated animals. That adriamycin cardiotoxicity may be mediated by histamine release is further supported by the fact that the morphological signs of this cardiomyopathy are similar to histamine-induced myocardial damage [16].

This study has demonstrated that an important histamine release can be produced also *in vitro* in rat peritoneal cells treated with adriamycin, and that the intraperitoneal injection of this drug in the mouse, induces a massive mast cell degranulation indicating release of histamine and possibly other substances such as serotonine. Pretreatment with high doses of the widely used solvent polyethylene glycol 400, significantly limited adriamycin associated cardiotoxicity [5, 6] and reduced histamine release from mast cells *in vivo* and *in vitro*.

The concentrations of polyethylene glycol 400 required to inhibit histamine release from mast cells *in vitro* and *in vivo* are extremely high, however similar concentrations are required also for other biological activities of these substances, such as induction of cell fusion [17], enhancement of *in vitro* lymphocyte stimulation [18] and antibacterial effect [19]. On the other hand, Magnusson *et al.* [7] have demonstrated that the spontaneous and polymyxin B-induced release of histamine is par-

tially inhibited by very low concentrations of polyethylene glycols; nevertheless this discrepancy could be ascribed to differences between the substances employed as secretagogues and to differences in the experimental procedure. In particular, about 98% pure mast cells were used by Magnusson *et al.* [7], whereas our experiments were conducted without purifying mast cells, since it has been shown [9, 10] that mast cells are the only source of histamine in the population of rat peritoneal cells.

It is interesting to note that polyethylene glycol 600 and 6000 can also inhibit the myeloperoxidase-mediated secretion of serotonine from rat peritoneal mast cells [20]; in fact it has been suggested that adriamycin induced cardiotoxicity is mediated not only by histamine, but also by other vasoactive substances [2–4], including serotonine.

It is not possible, as yet, to determine at what stage polyethylene glycol 400 is active in limiting histamine release *in vitro* and *in vivo*; however these findings provide a stimulus to test the activity of this solvent on the release of histamine induced by other drugs or chemicals or by immunological mechanisms.

Finally, the data presented in this study, those already published [5, 6] on the effects of polyethylene glycol 400 on adriamycin cardiotoxicity and those of other Authors [2–4] implicating histamine in the pathogenesis of acute, subacute and chronic adriamycin cardiotoxicity, makes inhibition of histamine release the most likely mechanism of the protective action of polyethylene glycol 400.

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