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Effect of cisplatin and *cis*-platinum (II) phosphonate complex on murine mast cells

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Abstract

We investigated the effect of two *cis*-platinum (II) complexes, *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) and *cis*-$[PtCl_2(4\text{-pmpe})_2]$ (4-pmpe stands for diethyl 4-pyridylmethylphosphonate), which was recently synthetized in our laboratory, on murine mast cells. We noticed that both tested compounds were able to evoke histamine release from murine mast cells. The histamine secretion was dependent on the concentration of compound and on the time and temperature of the reaction. It was also dependent on metabolic energy (the reaction was diminished in a medium without glucose and abolished in the presence of 2-deoxyglucose). The results indicate that *cis*-platinum (II) complexes activate mast cells to secrete histamine via a non-cytotoxic, active secretory process.

Keywords: *cis*-Platinum (II) phosphonate complex; Cisplatin; Murine mast cell; Histamine release

1. Introduction

It is well known that the therapeutic use of many drugs can be accompanied by the appearance of several side effects. Among them pseudoallergic drug reactions are very dangerous for patients, especially when they proceed as anaphylactic reactions (Anderson and Adkinson, 1987; DeSwarte, 1985). It is now established that some drugs cause anaphylactic reactions by immunological activation of mast cells and basophils (i.e. via specific immunoglobulin E (IgE) antibodies); however, some of them can act in a non-immunological direct way (Erffmeyer, 1992; Landry et al., 1992) and thus cause the release of many cellular mediators with a wide spectrum of biological activities (Schwartz, 1994).

It has been observed that many antineoplastic drugs can mediate hypersensitivity, anaphylactic reactions. In some cases these reactions are mediated by specific IgE antibodies but it seems that antineoplastic drugs often act in a specific, but non-immunological way (Botana et al., 1992a; Lagunoff et al., 1983; Weiss, 1982). However it should be pointed out that some of these drugs can also exert direct cytotoxic effects on cells.

The aim of our study was therefore to examine if well-known antineoplastic drug – cisplatin – and another newly synthesized complex – *cis*-dichlorobis(diethyl 4-pyridylmethylphosphonate) platinum (II) (*cis*-$[PtCl_2(4\text{-pmpe})_2]$) (Ochocki, 1994) – are able to exert a direct action on mast cells. Our experiments were done on peritoneal murine mast cells, which are widely considered to be a suitable model for pharmacological studies. The action of the investigated compounds on mast cells was estimated by measuring the release of histamine.

2. Materials and methods

2.1. Chemicals

All reagents and solvents used to prepare the compounds were of analytical grade. Other chemicals were purchased from Sigma Chemical Co.

2.2. Preparation of the complexes

cis-$[PtCl_2(NH_3)_2]$ was prepared from $K_2[PtCl_4]$ by using the method described by Dhara (1970). Diethyl 4-pyridylmethylphosphonate (4-pmpe) was prepared as previously described (Ochocki et al., 1992) and was purified

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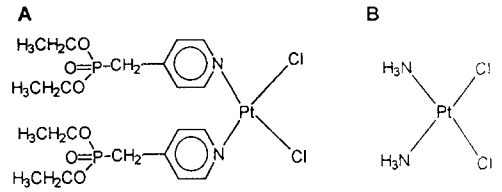

Fig. 1. The chemical structures of the *cis*-platinum (II) complexes used in the experiments. (A) *cis*-$[PtCl_2(4\text{-pmpe})_2]$; (B) *cis*-$[PtCl_2(NH_3)_2]$.

by distillation under reduced pressure (Fig. 1). *cis*-Dichlorobis(diethyl 4-pyridylmethylphosphonate) platinum (II) (*cis*-$[PtCl_2(4\text{-pmpe})_2]$) was prepared as follows: to a stirred solution of 4-pmpe (0.458 g, 2 mM) in methanol (2 ml) a solution of $K_2[PtCl_4]$ (0.415 g, 1 mM) in water (5 ml) was added dropwise. The yellow precipitate formed was washed with water and methanol and dried under vacuum over P_2O_5. The yield was 0.488 g (62%). All compounds were analysed by using high performance liquid chromatography techniques (HPLC) to ensure that the products were free of $K_2[PtCl_4]$ starting materials. Compounds were considered to be pure when less than 0.1% impurity was detected by HPLC.

2.3. Mast cell isolation

Female BALB/c mice 7–9 weeks of age were used in all experiments. The mast cells were collected from the peritoneal cavity by lavage with 2 ml of medium containing 117.6 mM NaCl, 3.48 mM KCl, 1 mM $CaCl_2$, 0.67 mM $MgSO_4 \times 7\ H_2O$, 5.6 mM glucose, 50 mM Hepes, pH 7.4. After abdominal massage (90 s) the cell suspension was removed from the peritoneal cavity with a pipette. The cells were washed 3 times with the same medium by centrifugation (6 min, 1200 rpm). After being washed, the mast cells were counted and suspended in an appropriate volume of medium ($4–5 \times 10^5$ mast cells/ml). In each experiment cell viability was determined using the trypan blue dye technique. The unpurified cellular suspension contained 3–4% mast cells and the viability of these cells was 96–98%.

2.4. In vitro mast cell experiments

The suspension of the cells was divided into 90-μl aliquots and after equilibrium at 37°C for 5 min 10 μl of a solution of the test compound (*cis*-platinum (II) complex or compound 48/80) in an appropriate concentration, as specified in the results, was added. The incubation was carried out in a water bath at different temperatures and for different periods of time as stated in the results. In some experiments the medium was without glucose or without glucose but with 1 or 5 mM 2-deoxyglucose. The reaction was stopped by adding 0.9 ml of cold medium. Next the cell suspensions were centrifuged (6 min, 1200 rpm), and the supernatants were collected and decanted into other tubes for histamine determination. In every experiment appropriate controls for the determination of spontaneous histamine release in the absence of stimuli were executed.

2.5. Histamine-release assay

The histamine content was determined spectrofluorometrically in both the cell pellet (residual histamine) and in the supernatants (released histamine), omitting the extraction procedure (Shore et al., 1959). Histamine release was expressed as a percentage of the total cellular content of the amine after correction for the spontaneous release found in controls. The mean spontaneous histamine release was from 7 to 12%.

It should be pointed out that our additional control experiments showed that there was no interference of *cis*-platinum (II) complexes with the histamine determination by the spectrofluorometric method.

3. Results

3.1. The effect of the compounds on histamine release from murine mast cells

The ability of *cis*-$[PtCl_2(4\text{-pmpe})_2]$ and *cis*-$[PtCl_2(NH_3)_2]$ to induce histamine release from murine mast cells in vitro is presented in Fig. 2. In our experimental conditions both studied compounds were able to evoke histamine release from the mast cells in a concentration-dependent manner. Drug-induced histamine secretion was evoked with concentrations $> 10^{-13}$ M and reached a maximum rate at a concentration of 10^{-10} M. Higher concentrations of compounds caused lower histamine secretion. We noticed that *cis*-$[PtCl_2(NH_3)_2]$ caused less histamine release from mast cells than *cis*-$[PtCl_2(4\text{-}$

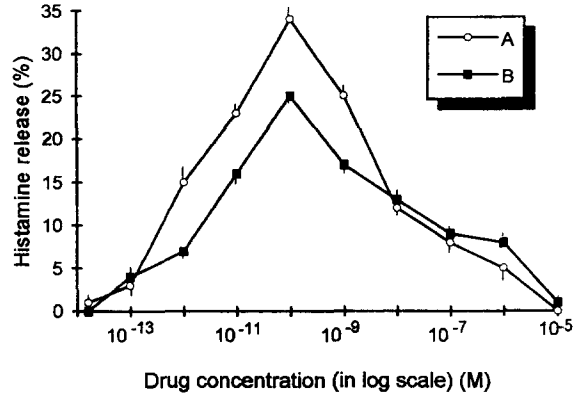

Fig. 2. Concentration-response curves for histamine release from murine mast cells evoked by *cis*-platinum (II) complexes. The mast cells were incubated with the compounds for 20 min at 37°C. The points are the means from 4 separate experiments ± S.E.M. (A) *cis*-$[PtCl_2(4\text{-pmpe})_2]$; (B) *cis*-$[PtCl_2(NH_3)_2]$.

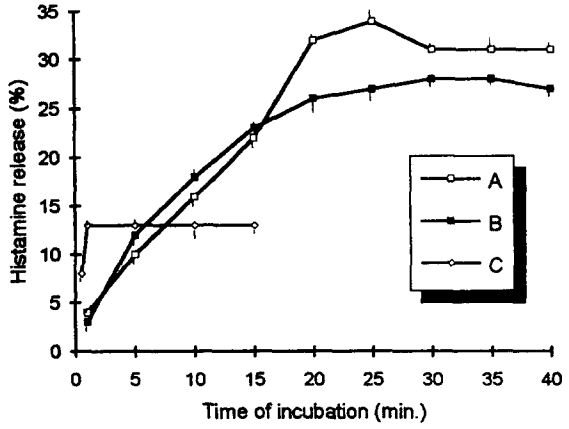

Fig. 3. Time course of histamine release from murine mast cells induced by *cis*-platinum (II) complexes and compound 48/80. The mast cells were stimulated at 37°C by (A) *cis*-$[PtCl_2(4\text{-pmpe})_2]$ at 10^{-10} M, (B) *cis*-$[PtCl_2(NH_3)_2]$ at 10^{-10} M, and (C) compound 48/80 at 1 μg/ml. The points are the means from 4 separate experiments ± S.E.M.

pmpe)$_2$] did (both drugs at concentrations 10^{-10} M) (24.6 vs. 34.0%).

3.2. The effect of time and temperature of the reaction on drug-induced histamine release from murine mast cells

Both compounds at a concentration of 10^{-10} M caused time-dependent histamine secretion (Fig. 3). The release of histamine was detectable 1 min after challenge (3.2% for *cis*-$[PtCl_2(4\text{-pmpe})_2]$ and 4.0% for *cis*-$[PtCl_2(NH_3)_2]$, with the maximum release occurring 20 min after challenge. At the same time compound 48/80 caused significant histamine release after 30 s of incubation and maximal levels were reached within 60 s of the challenge (Fig. 3). The release of histamine induced by *cis*-platinum (II) complexes was also temperature-dependent, with the maximum at 37°C for both studied compounds (Table 1).

3.3. The metabolic energy dependence of drug-induced histamine release from murine mast cells

In order to establish the dependence of the observed histamine release evoked by the drugs on glycolytic and oxidative cellular metabolism, the mast cells were incubated with the compounds in a concentration of 10^{-10} M for 20 min at 37°C in [A] complete medium with glucose, [B] glucose-free medium, or [C] glucose-free medium with 1 mM or 5 mM 2-deoxyglucose. In these experimental conditions we observed that the release of histamine was diminished (in a medium without glucose) to 40–42% of maximal release and abolished (in the presence of metabolic inhibitor – 2-deoxyglucose) to 8% (1 mM 2-deoxyglucose) and to 2% (5 mM 2-deoxyglucose) of maximal histamine release.

Table 1
The effect of temperature on histamine release evoked from murine mast cells by *cis*-platinum (II) complexes

Temperature of incubation (°C)	Percentage of histamine release induced by	
	(A)	(B)
4	0	0
25	12.0 ± 1.7	9.9 ± 1.0
37	37.9 ± 3.4	28.3 ± 3.0
45	3.7 ± 0.8	5.8 ± 0.6

[a]The mast cells were incubated for 20 min with the compounds at 10^{-10} M. Each value represents the mean from 4 separate experiments ± S.E.M. (A) *cis*-$[PtCl_2(4\text{-pmpe})_2]$; (B) *cis*-$[PtCl_2(NH_3)_2]$.

4. Discussion

Since the discovery of the antitumour activity of *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) (Rosenberg et al., 1969), a great number of new platinum complexes have been obtained and tested against various tumour systems. Two platinum (II) complexes are now widely used for the treatment of cancer, *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) and [Pt(II)-$(NH_3)_2$(CBDCA)] (*cis*-diammine (1,1-cyclobutanedicarboxylato) platinum (II) (carboplatin) (Reedijk, 1992). However, the search for new potent platinum complexes that possess a boader spectrum of antitumour activity, lower toxicity, lack of cross-resistance, and desirable physico-chemical properties continues. Recently, platinum (II) complexes which are linked to phosphonate ligands have been reported (Klenner et al., 1993). These compounds accumulate selectively in bone and represent a good tool for the treatment of bone malignancies and metastases. In our search for effective new platinum antitumour complexes we have reported on the synthesis of new complexes with the general formula *cis*-$[PtCl_2L_2]$ (L stands for diethyl 2-, 3-, and 4-pyridylmethylphosphonate ligands) (Ochocki, 1994) and preliminary data for their antitumour activity against solid Sarcoma-180 (Ochocki et al., 1994). Considering the possibility that they may elicit some undesirable effects, we studied the action of two platinum compounds – *cis*-$[PtCl_2(4\text{-pmpe})_2]$ and *cis*-$[PtCl_2(NH_3)_2]$ – on mast cells to determine whether or not they can cause the release of histamine.

Our studies showed that both platinum (II) complexes were able to cause histamine release from murine mast cells. The histamine release was dependent on the concentration of compound used; however, we noticed that at higher concentrations of the compounds ($> 10^{-10}$ M), the response of the mast cells was abolished. The secretion of histamine was dose- and temperature-dependent. It was also dependent on glycolytic and oxidative cellular metabolism. Taken together, it may be concluded that both tested platinum (II) complexes cause the release of histamine from mast cells via an active secretory process.

It has been noticed that occasionally the therapeutic use of antineoplastic drugs is accompanied by the appearance of side effects due to the release of histamine (Unverfert et

al., 1982; Weiss, 1982). It has also been shown that some antineoplastic agents elicit histamine release from rat mast cells in vitro via a non-immunological mechanism by direct activation of mast cells (Botana et al., 1992a, b; Riegel et al., 1982). Our experiments have shown that two platinum (II) complexes – cisplatin and *cis*-$[PtCl_2(4\text{-}pmpe)_2]$ – can cause the secretion of histamine from murine mast cells in vitro.

The mechanism of action of platinum (II) complexes on mast cells is unknown. It may be however supposed that the activation of mast cells is not mediated by specific receptors and probably involves G-proteins, as in the case of basic releasers (Mousli et al., 1990a, b). However, it should be pointed out that in the case of histamine release induced by compound 48/80, the reaction was very fast and a maximal response was achieved within 1 min. Further experiments will be performed to determine the nature of the stimulation of mast cells by platinum (II) complexes and will deal with related compounds like *cis*-dichlorobis(diethyl 2-pyridylmethylphosphonate) and *cis*-dichlorobis(diethyl 3-pyridylmethylphosphonate) platinum (II) complexes.

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