IMMUNOBIOLOGICAL ACTIVITY OF COORDINATION COMPOUNDS OF PLATINUM FORMING COMPLEXES WITH IMMUNOGLOBULIN FRAGMENTS

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Coordination compounds of platinum are nowadays regarded as a most promising group of antitumor agents, whose mechanism of action has not been fully explained [2]. We know that platinum compounds [11] are actively bound in vivo with various biological ligands, including blood plasma proteins [6], which play an important role in the maintenance of homeostasis [3].

Research aimed at studying interaction of platinum reagents with immunoglobulins and with their antigen-binding Fab-fragments, and the effect of complex formation on their biological activity, is therefore of considerable interest. A particularly valuable feature of such research is the possibility of construction of metal-containing biostimulators and immunotoxins.

The aim of this investigation was to study immunomodulating activity and hematotoxicity of two coordination compounds of platinum [2] with a broad spectrum of biological activity: *cis*-dichlorodiaminoplatinum (*cis*-DDP) and a new promising compound dichloro-N,N,N,N-tetrakis(2-aminoethyl)-1,6- hexamethylenediamino-*bis*-platinodichloride with the general formula (Pt_2AmCl_4) [1], and their complexes with Fab-fragments of nonspecific human immunoglobulin (IgG). The Fab-fragments were obtained by the known method of controlled enzymic hydrolysis with papain [4].

EXPERIMENTAL METHOD

Fab-fragments, used in a concentration of 5 mg/ml, were modified with the aid of platinum reagents in the ratio Pt:protein by weight of 1:20 in 0.05 M phosphate buffer (pH 7.4) for 24 h at 37°C. Excess of unreacted platinum reagents was removed by chromatography on a column of Sephadex G-25, fine ("Pharmacia," Sweden), using the above phosphate buffer as eluent.

The number of platinum groups bound with protein molecules was determined by neutron-activation analysis with radiochemical concentration of the detected element (^{197}Pt) [5]. The content of platinoids was found to be on average three molecules to one protein molecule.

Investigation of the immunomodulating activity of the platinum-globulin complexes, of the initial immunoglobulin fragments, and of the platinum reagents (as the control) was investigated in a standard microtest version of the blast transformation reaction of human lymphocytes, stimulated to proliferate by the mitogen phytohemagglutinin (PHA, 10 μg/ml), based on incorporation of ^3H-thymidine. Radioactivity was measured on a Mark III scintillation counter (USA). The cytotoxic effect of the preparations was studied on a short-term suspension culture of healthy human lymphocytes, cultured in medium RPMI-1640 with 10% embryonic calf serum ($2 \cdot 10^6$ cells/ml); their viability was estimated by staining with trypan blue after incubation for 24 and 48 h.

The hemotoxicity of the platinum reagents also was stained in vivi on BALB/*c* mice receiving a single intraperitoneal injection in a dose close to the maximally tolerated dose (10 mg/kg).

The results of five or six experiments were subjected to statistical analysis by the standard method.

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TABLE 1. Antiproliferative Activity of Modulating Effect of Pt_2AmCl_4 and its Complexes with Immunoglobulin Fragments in Blast Transformation Reaction of Human Lymphocytes Reflected in Incorporation of ^3H-Thymidine (number of counts per minute); $M \pm m$

PHA	PHA + Fab (200 μg/ml)	PHA + Pt_2AmCl_4 (in μg/ml)			PHA + Pt_2AmCl_4 complex*
		0,5	10	200	10 μg/ml
19 963±1502,5	18 449,2±1354,8	24 025,2±1892,5	6726,3±1114,6	597,0±158,3	25 174,3±2147,2

Legend. Asterisk indicates concentration of platinum.

EXPERIMENTAL RESULTS

TABLE 2. Number of Peripheral Blood Cells (in 1 μl) in BALB/*c* Mice 24 h after a Single Intraperitoneal Injection of *cis*-DDP (1), $PtAmCl_4$ (2), and their Complexes with Immunoglobulin Fragments ($M \pm m$)

Reagent	Erythrocytes, $\times 10^6$/μl	Leukocytes, $\times 10^3$/μl
cis-DDP	**6,5±0,4**	**5,2±1,4**
Conjugate 1	6,6±0,18	13,1±2,54
Pt_2AmCl_4	**6,76±0,32**	**5,7±1,6**
Conjugate 2	6,7±0,13	11,3±1,34
Background	7,0±0,2	12,3±2,5

A comparative study of the biological activity of *cis*-DDP and its complexes with immunoglobulins on immunocompetent cells in vitro revealed the presence of a suppressor effect of cisplatin, the magnitude of which depended on the dose of the compound. For instance, with a dose of *cis*-DDP of 10 μg/ml the level of incorporation of the radioactive label into the cells fell from 19,963 ± 1502.2 cpm compared with the control to 1186.4 ± 217.2 cpm in the experiment ($p < 0.001$). However, the addition of a complex of *cis*-DDP with protein to the cell suspension in a dose equivalent for platinum content was not accompanied by any statistically significant suppression of cell proliferation ($p > 0.5$). In this case, there was no cytotoxic effect: the percentage of viable cells after incubation of the immune preparations and conjugates for 24 h was 91-94% (94.5% in the control).

Response of lymphocytes to PHA during isolated exposure to immune preparations showed no substantial change ($p > 0.5$).

A similar rule was found in the case of Pt_2AmCl_4. It was found that this reagent, in a concentration of 10 μg/ml, reduced PEW-induced blast transformation statistically significantly ($p < 0.001$), but with higher concentrations, $PtAmCl_4$ possessed a total mitostatic suppressor action. Meanwhile the addition of its complexes with proteins to the cell culture was accompanied in some cases by stimulation of the immune response (by 20-45% of the background), but without any cytotoxic side effects.

It will be noted that $PtAmCl_4$ in lower concentrations (of the order of 0.5 μg/ml) also had a certain immunostimulating effect (Table 1).

Analysis of the hematologic data showed that the platinum compounds, if injected into experimental mice in subtoxic doses (10 mg/kg), induced the development of a short-term leukodepressive effect, which was most marked on the 2nd-3rd day of the experiment (the leukocyte count was reduced by 35-54% below the background level). However, after treatment with the platinum-immunoglobulin complexes, no leukopenic effect was observed in the experimental mice (Table 2). Thus the tendency for the cytostatic activity of platinum reagents bound with immunoglobulins to decrease, discovered in experiments in vitro, is also found in vivo.

The results of these investigations, taken as a whole, are evidence, first, of reduction of the antiproliferative activity and cytotoxicity of coordination compounds of platinum [2] in the form of complexes with immunoglobulin preparations; second, they show that limited addition of platinum to immunoglobulin fragments, in the case of $PtAmCl_4$, increases their

immunomodulating activity; this paves the way for targeted addition of reagents containing reactive platinum groups to immunologic preparations, with the aim of obtaining biologically active complexes.

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EFFECT OF STIMULATION OF OPIOID RECEPTORS ON LYMPHOCYTE FUNCTION IN VITRO

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The list of mediators of neuroimmune interactions includes opioid peptides (enkephalins, endorphins, dynorphins, etc.) which participate in the regulation of emotional responses, the functioning of peripheral organs, development of the adaptation syndrome, and potentiation of immune responses [1]. In particular, Met-enkephalin has been shown to stimulate activity of natural killer cells [5], EA-rosette formation [7], antibody-dependent cellular cytotoxicity [6], and leukocyte migration [10].

The aim of this investigation was to study the effect of Met-enkephalin on spontaneous adhesion and on phytohemagglutinin- (PHA-) stimulated proliferative activity of healthy human lymphocytes in the blast transformation reaction (BTR) in vitro, using naloxone, a blocker of opioid receptors.

EXPERIMENTAL METHOD

Cells were isolated and spontaneous adhesion of healthy human peripheral blood lymphocytes were studied by the method described previously [2, 3]. A suspension of healthy human lymphocytes in a concentration of $2 \cdot 10^6$ cells/ml in medium 199 with 15% embryonic calf serum, inactivated by heating at 56°C for 30 min, in a volume of 0.1 ml, 0.05 ml of a solution of Met-enkephalin ("Serva," West Germany) and, if necessary, 0.05 ml of naloxone solution ("Hoffmann-LaRoche, USA) were added to the wells of 96-well flat-bottomed plastic panels ("Falcon Plastics," USA). The panels were incubated at 37°C in an atmosphere of 5% CO_2 in a humid chamber for 1.5 h. Cells nonadherent to the bottom of the wells were then separated and counted, as described previously [2, 3]. The reaction was assessed by the usual formula for studying inhibition of lymphocyte adhesion (ILA):

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