STUDY OF SOME PLATINUM (II), PALLADIUM (II), AND RHODIUM (III) COORDINATION COMPOUNDS AND THEIR INFLUENCE ON HUMORAL IMMUNE RESPONSE OF WHITE MICE

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الخلاصة :

تمت دراسة المركبات التناسقية الناتجة عن تفاعل أيونات البلاتين ثنائي التكافؤ ، والبلاديوم ثنائي التكافؤ، والروديوم ثلاثي التكافؤ على التوالي ، مع ٢ ، ٦- ثنائي أمين البريدين من حيث فعاليتها في علاج السرطان.

دلت نتائج هذه الدراسة على أن مركبات البلاتين والبلاديوم والروديوم ذات فعالية عالية في تقوية المناعة في جسم الحيوان ضد السرطان ، وقد كانت مركبات البلاتين أكثر هذه المركبات فعالية في تعزيز القدرة على إفراز الأجسام الحيوية المضادة في جسم الحيوان . وقد أظهرت مركبات البلاديوم والروديوم فعاليات متفاوته ، اختلفت باختلاف الوقت وظروف حقن المواد في جسم الحيوان .

ABSTRACT

The coordination compounds of platinum(II), palladium(II), and rhodium(III) with 2,6-diaminopyridine (DAP) have been investigated as potential anti-tumor compounds. 2,6-Diaminopyridine complexes of Pt, Pd, and Rh exhibit significant effects on the humoral immune response in the animal system. The Pt complex was most consistent in enhancement of antibody forming cells since all groups injected with the complex showed a positive response. Both Pd and Rh showed different antimitogenic activities but the effect depended on both the time factor and the conditions of injection.

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1. INTRODUCTION

Rosenberg [1, 2] suggested a possible mode of action of the Pt anti-tumor drugs since most of these complexes were found to be immune-suppressive agents. His hypothesis was that transformed cells produced new antigens at the cell surface which generated a host immune reaction leading to cell destruction. However, if some transformed cell types masked the strong antigens with the relatively much weaker antigens such as nucleic acids, then they would escape the host immune surveillance and form a tumor clone. An extension of this hypothesis was that the platinum complexes enhanced the antigenecity of tumor cells by forming primary lesions in the DNA. These lesions would lead to the disruption of the antigen mask and exposure of the strong antigen on the cell surface so that even the weakened immune competence of the host can still amount an adequate response and selectively destroy the tumor tissue. The synthesis and antimitogenic properties of some 2,6-diaminopyridine (DAP) complexes of Pd(II), Pt(II), and Rh(III) have been reported [3]. More recently platinum complexes of 1,2-diaminocyclohexane have been investigated at the early clinical trial stages [4]. Diaminopyridine complexes have also been investigated with respect to their effect on sodium and calcium retention in rat kidneys [5]. The effect of biologically active coordination compounds on the cellular immune response has been investigated through different approaches. One such approach was based on the fact that protein tyrosine phosphorylation appears to be a major intracellular signalling event that mediates cellular responses. Cisplatin (*cis*-dichlorodiamineplatinum(II)) was therefore examined with respect to its effect on tyrosine phosphorylation in macrophages [6]. It did enhance phosphorylation.

The effect of DAP complexes on the cellular immune response was measured, *in vitro*, by the uptake of radioactive thymidine [3]. Although most of these complexes were found to inhibit DNA synthesis, it was observed that Rh(III) complexes were found to extremely enhance thymidine uptake. This may indicate, in view of Rosenberg's hypothesis, that Rh (III) may prove to have high anti-tumor activity. In view of their low toxicity [3], it looked feasible to investigate the biological activity of Pt(II), Pd(II), and Rh(III) complexes using a different probe, namely; measuring their influence on the humoral immune response of white mice.

2. RESULTS AND DISCUSSION

Solubility Studies

Solubility of each of the complexes in water was determined by the UV and visible spectra for different dilutions of its saturated solution in distilled water. The wavelength corresponding to maximum absorption (λ_{max}) was recorded for all complexes and the molar absorptivities $\varepsilon\lambda_{max}$ were calculated from Beer's Law. The results are shown in Table 1.

The values of the solubility at room temperature in mg/ml and molar are listed in Table 2.

Solubility of the famous *cis*-$Pt(NH_3)_2Cl_2$ is included in Table 2 for comparison. It can be seen that the Pt-DAP complex is more soluble.

Table 1. λ_{max} (nm) and Molar Absorptivities for 2,6-Diaminopyridine Complexes.

Compound	λ_{max} (nm)	$\varepsilon^{1cm}_{\lambda max}$ (Absorbance/cm.molar)
Pd (DAP) Cl_2. H_2O	336	3580
Pd $(DAP)_2Cl_2$	336	9500
Pt $(DAP)_2$ Cl_2.2HCl	240; 335	19200; 19000
Rh (DAP) Cl_3. $2H_2O$	295	4400

Biological Studies

The notion that cells can effect immune responses either directly by proliferation of lymphocytes or through antibody production has gained considerable interest in cancer chemotherapy research. The immune responses, whether cellular or humoral, are the basic immune reactions against immunogen, antigens, and disease.

We have previously reported on the effect of Pd, Pt, and Rh complexes on the cellular immune responses [3]. The present work deals with the determination of adjuvant activity of these complexes on the immune response to antigen, sheep blood red cells (S-RBC) in mice.

Antibody Plaque Formation

Activity was tested by the Plaque Assay Technique. The counts of antibody plaque formed by the spleen cells suspension obtained from mice injected with 0.5 ml of 1.7×10^{-3} M $Pt(DAP)_2Cl_2.2HCl$ and 0.1 ml of 20% sheep S-RBC are listed in Table 3.

The counts of plaque formed by spleen cell suspension obtained from mice injected with Pd - or Rh(DAP) complexes and S-RBC are listed in Table 4. The dosage of Pd and Rh complex used was 5.8 and 6.7 mg/kg. The dosage of the mitogen S-RBC used was 0.1 ml of 20% S-RBC for each mouse.

Nucleated White Cells in Spleen Cell Suspension

Microscopic examination of the spleen suspension of mice injected with 0.5 ml of $Pt(DAP)_2Cl_2.2HCl$ and 0.1 ml 20% S-RBC obtained after washing by three centrifugations and resuspensions in Hank's medium showed the presence of nucleated white cells. The counts of these cells are listed in Table 5.

Similarly, the counts of nucleated white cells in cell suspension of mice injected with 0.5 ml $Pd(DAP)Cl_2.H_2O$ and $Rh(DAP)Cl_3.2H_2O$ and 0.1 ml of 20% S-RBC are listed in Table 6.

Table 2. Solubility of Pd(II), Pt(II), and Rh(III) Complexes in Water.

Compound	Solubility	
	mg/ml	M
Pd (DAP) $Cl_2.H_2O$	0.348	1.14×10^{-3}
Pd $(DAP)_2Cl_2$	0.262	6.7×10^{-4}
Pt $(DAP)_2$ $Cl_2.2HCl$	4.810	8.63×10^{-3}
Rh (DAP) $Cl_3.2H_2O$	0.432	1.14×10^{-3}
cis-Pt $(NH_3)_2Cl_2$	25.2	8.4×10^{-3}

Table 3. Antibody Plaques Counts of Spleen Cell Suspension Obtained from Mice Injected with Platinum DAP Complex and S-RBC.

Group	Spleen	Plaque		
		3 Days	6 Days	12 Days
Control	3	1	17	6
S-RBC	3	1	21	43
Pt + S-RBC	3	3	58	42
Pt (24 hours) + S-RBC	3	1	47	65
Pt + S-RBC (24 hours)	3	1	87	61

Table 4. Antibody Plaques Counts of Spleen Cell Suspension Obtained from Mice Injected with Palladium and Rhodium DAP Complexes and S-RBC.

Group	Spleen	Plaque		
		3 Days	6 Days	12 Days
Control	3	6	8	9
S-RBC	3	3	30	15
Pd + S-RBC	3	35	24	20
Rh + S-RBC	3	35	27	28
Pd (24 hours) + S-RBC	3	40	37	24
Rh (24 hours) + S-RBC	3	44	16	28
Pd + S-RBC (24 hours)	3	60	28	25
Rh + S-RBC (24 hours)	3	66	66	20

Table 5. Nucleated White Cell Counts Spleen Cell Suspension Obtained from Mice Injected with $Pt(DAP)_2Cl_2.2HCl$ and S-RBC.

Group	Nucleated White Cells	
	6 Days	12 Days
Control	610	83
S-RBC	760	483
Pt + S-RBC	400	275
Pt (24 hours) + S-RBC	840	338
Pt + S-RBC (24 hours)	300	338

Table 6. Nucleated White Cell Counts Spleen Cell Suspension Obtained from Mice Injected with $Pd(DAP)Cl_2.H_2O$ and $Rh(DAP)Cl_3.2H_2O$ and S-RBC.

Group	Nucleated White Cells		
	3 Days	6 Days	12 Days
Control	38	160	152
S-RBC	53	450	180
Pd + S-RBC	470	629	150
Rh + S-RBC	245	1098	120
Pd (24 hours) + S-RBC	80	539	110
Rh (24 hours) + S-RBC	71	57	150
Pd + S-RBC (24 hours)	245	450	250
Rh + S-RBC (24 hours)	1390	687	160

Agglutination Titers

The agglutination titer is the highest dilution of serum which will give a positive indication of the presence of antibodies. Table 7 lists the titer of blood serum of mice injected with 0.5 ml of platinum complex and 0.1 ml of 20% S-RBC. Table 8 lists the titer of blood serum of mice injected with 0.5 ml of Pd or Rh complex and 0.1 ml of 20% S-RBC. The titer gave a negative test for blood serum of 3 days.

Plaques per Million Nucleated Spleen Cells

The number of plaques obtained is proportional to the number of antibody producing cells plated in Agar. Each plaque is due to the activity of an individual cell. The plaque count is multiplied by ten to determine the total number of antibody producing cells in the spleen cell suspension.

$$\text{Plaques } /10^6 \text{ nucleated spleen cells} = \frac{\text{Number of plaques} \times 10 \times 10^6}{\text{White cells} \times 200 \times 1000}.$$

The number of plaques per million nucleated cells is determined for spleen cell suspension of Pt (DAP) complex and is listed in Table 9. The number of plaques per million nucleated cells is determined for spleen cell suspension of Pd and Rh complexes and is listed in Table 10.

Table 7. Agglutination Titer of Blood Serum of Mice Injected with Pt Complex and S-RBC.

Group	Titer		
	3 Days	6 Days	12 Days
Control	Zero	Zero	Zero
S-RBC	Zero	1/640	1/640
Pt + S-RBC	1/80	1/320	1/640
Pt (24 hours) + S-RBC	1/20	1/320	1/640
Pt + S-RBC (24 hours)	1/80	1/1280	1/640

Table 8. Agglutination Titer of Blood Serum of Mice Injected with Pd and Rh Complexes and S-RBC.

Group	Titer		
	3 Days	6 Days	12 Days
Control	Zero	Zero	Zero
S-RBC	Zero	1/80	1/160
Pd + S-RBC	Zero	1/80	1/160
Rh + S-RBC	Zero	1/80	1/160
Pd (24 hours) + S-RBC	Zero	1/80	1/320
Rh (24 hours) + S-RBC	Zero	1/80	1/160
Pd + S-RBC (24 hours)	Zero	1/640	1/160
Rh + S-RBC (24 hours)	Zero	1/160	1/640

Evaluation of the Activity of Various DAP Complexes

The results indicate that considerable adjuvant effect was shown by the complexes and significant enhancement of immune response was achieved.

Platinum Complexes. Table 9 shows that the number of plaque-forming or antibody-producing cells was significantly enhanced for all groups treated with the Pt complex. Maximum immune enhancement occurred when the compound was given 24 hour prior to the antigen. The response was stronger for the 12 days spleen cell suspension than for the 6 days suspension. This is probably due to a possible adjuvant effect of the Pt complex in enhancing the differentiation of antibody producing cells.

However, the serum-antigen-S-RBC antibody titer showed no significant effect. The agglutination titer, which has a less sensitive response to antigen than plaque-forming response, showed suppression for blood serum obtained after 6 days injection. This suggests that the effect of the compound had not reached the serum of the mice.

Table 9. The Number of Plaques per 10^6 Nucleated Spleen Cells of Spleen Cell Suspension of Mice Injected with Pt Complex and S-RBC.

Group	Plaques/10^6 Nucleated Cells	
	6 Days	12 Days
Control	2.5	2.5
S-RBC	3.2	4.3
Pt + S-RBC	3.4	7.5
Pt (24 hours) + S-RBC	5.9	9.6
Pt + S-RBC (24 hours)	14.5	16.5

Table 10. The Number of Plaques per 10^6 Nucleated Spleen Cells of Spleen Cell Suspension of Mice Injected with Pd and Rh Complexes.

Group	Plaques/10^6 Nucleated Cells	
	6 Days	12 Days
Control	2.5	2.5
S-RBC	3.2	4.3
Pd + S-RBC	1.9	6.6
Rh + S-RBC	2.2	11.6
Pd (24 hours) + S-RBC	3.5	9.0
Rh (24 hours) + S-RBC	14.5	11.0
Pd + S-RBC (24 hours)	3.1	5.3
Rh + S-RBC (24 hours)	4.8	6.4

Palladium Complexes. The plaque-forming assay showed no significant enhancement of the immune response to the antigen S-RBC after 6 days of injection with Pd complex. The response increased after 12 days and the enhancement was maximized by injecting the complex 24 hours after the antigen. This was probably due to the fact that the antigen induced the host's natural immune response and that the compound, injected 24 hours later, acted on the antibody-producing cells by enhancing their differentiation. The agglutination in serum antibody response showed little effect for blood serum obtained from mice after 6 days and 12 days of injection.

Rhodium Complexes. No enhancement of antibody plaque-forming cells was shown after 6 days of injection of the Rh complex for the group of mice injected 24 hours after S-RBC antigen injection. Maximum enhancement of the immune response for mice injected with Rh complex occured after a time limit of 12 days for the group injected with the complex simultaneously with the antigen.

Agglutination titer showed no effect for the 6 days and 12 days serum.

Specificity of Immune Responses. The present work showed that Pt, Rh, and Pd complexes of 2,6-diaminopyridine exhibit significant effect on the humoral immune response in the animal system. The platinum complex was most consistent in enhancement of antibody forming cells since all groups injected with the complex showed positive response.

Both palladium and rhodium showed different antimitogenic activities but the effect depended on both: time factor and conditions of injection.

The concept of specificity in the immune response is inherent in the immune reaction of host animals and recommends itself as a dominant component in selective antibody–antigen complex. It is difficult at this stage to see how the various complexes tested for humoral immune response, successfully stimulate a specific immune attack upon a certain antigen. It is possible to extend the work and test the effect of these complexes on a spectrum of tumors to find out the relative selectivity and specificity towards these tumors.

Since the quantitative enhancement in antibody-producing cells, was observed for mice injected with Pt, Pd, and Rh complexes of 2,6-diaminopyridine, relative to the control, it can be said that the effect of these complexes clearly involves the B cells.

3. EXPERIMENTAL

Preparation of Coordination Compounds

Palladium(II), Pt(II), and Rh(III) coordination compounds of 2,6-diaminopyridine (DAP); namely, $Pd(DAP)Cl_2.H_2O$, $Pt(DAP)_2Cl_2.2HCl$ and $Rh(DAP)Cl_3.2H_2O$ were prepared by a literature method [3].

Solubility Measurement

The solubility of each of the coordination compounds in water was determined according to the following procedure:

A saturated solution of each of the metal complexes was prepared by dissolving a few crystals in 10 ml of distilled water. The solution was homogenized by sonification and then filtered through a 0.45 μ pore size millipore filter. Visible and ultra-violet spectra were run for the saturated as well as for diluted solutions. The wave length λ_{max} was recorded; and from Beer's law, molar absorptivities were calculated.

Biological Tests

The effect of the complexes on the humoral immune response was determined by Jerne's experimental technique [7]. The method involves counting of anti-body producing cells of a spleen cell suspension of an animal immunized against the antigen sheep red blood cells (S-RBC). The antibody diffusing from an antibody-producing cell reacts specifically with the antigen. When the complement is added, these cells lyse forming small plaques, thus identifying individual antibody producing cells.

Materials

Hank's balanced salt solution (HBSS) [8] was prepared according to Hank's formula [9]. The solution was stabilized by adjustment to pH 7.0 with $NaHCO_3$ during preparation.

Base layer petris, Ager tubes, and Vernol buffer were prepared by normal procedures. Guinea pig serum was reconstituted with 2 ml distilled water. One ml of the serum was added to 7 ml distilled water and diluted 10 fold with Vernol buffer.

Testing Procedures

Protocol of Animal Study. The biological tests for anti-mitogenic activity of metal complexes of 2,6-diaminopyridine were carried out on four-months-old white mice. Different injection schedules were given to the mice which were divided into five groups:

Group I : Control (no injection was given)

Group II : S-RBC (0.1 ml of 20% S-RBC was injected)

Group III : S-RBC + Drug (0.1 ml of 20% S-RBC and 0.5 ml of one of the metal complexes were injected simultaneously)

Group IV : S-RBC + Drug (24 hours later)
The antigen (0.1 ml) of 20% S-RBC was injected and 0.5 ml of the drug was injected 24 hours later.

Group V : Drug + S-RBC (24 hours later)
The drug (0.5 ml) was injected and 20% S-RBC (0.1 ml) was given 24 hours later.

Each group consisted of nine mice. Of these, three mice were sacrificed every 3 day, 6 days, and 12 days, respectively.

Antibody Plaque Counting. The procedure of Jerne described elsewhere [7] was followed.

Agglutination Titer. The readings were taken according to a literature procedure [10, 11]. White-cell counting was done for two drops of the diluted (with 20% acetic acid) spleen cell suspension using a microscope.

Instrumental

Ultraviolet and visible spectra were recorded using a Perkin Elmer 202 spectrophotometer. pH measurement were done using a pH meter 29.

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