Vol. 104, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1982 Pages 121-125

BIOLOGICAL ACTIVITY OF METAL COMPLEXES.

V : Influence of Pd(II),Pt(II),and Rh(I) on the Macrophages Chemotaxis.

MICHELE ARESTA , MARIA DE FAZIO
Istituto di Chimica Generale ed Inorganica,Via Amendola 173,70126 BARI,Italy,

RUGGERO FUMARULO, DOMENICO GIORDANO, ROMEO PANTALEO, SILVANA RICCARDI
Istituto di Patologia Generale,I Cattedra,Piazza Giulio Cesare,70124 BARI,Italy.

Received October 7, 1981

The influence of some Pd(II),Pt(II) and Rh(I) complexes on the macrophages chemotaxis has been evaluated in the range of temperature 0 - 37 °C and at various concentrations. All the complexes cause a chemotactic defect.The experimental results suggest that at 0 °C the complexes interact essentially with the membrane of the macrophages,while at 37 °C,more likely,they interact with nuclear DNA.

Introduction

Recently we have reported that cis-$Pt(NH_3)_2Cl_2$ | CISPLATIN|,entered in human therapy as a drug specifically active against testicular and ovarian cancers,depresses the macrophages chemotaxis |1|.As it seems ascertained that the immune system can play an important role in the pathogenesis of tumor growth |2| and it has been pointed out that the macrophages are involved in such a cell-mediated response to carcinogenesis |3|, we have investigated the influence on the macrophages chemotaxis of some Pd(II),Pt(II) and Rh(I) complexes which show significant biological activity (for example inhibit the DNA,RNA and protein synthesis |4| or induce E.Coli elongation |5|)in order to get informations on the mode of action of the drugs and find out correlations between the structures of the complexes and their biological activity.

We wish to report here the results of our studies on the modification of the macrophages chemotaxis caused by $\{Pt|S\text{-}(CH_2)_3Cl|_2\}_6$ (Pt_{10});Pt(2,6-diallylaniline)Cl_2 (Pt_{11}); $Pt_2|Se{=}P(NMe_2)_3|_2Cl_4$ (Pt_{12});Pt_2(julolidine)$_2Cl_4$ (Pt_{13});trans-$Pd|Se{=}P(NMe_2)_3|_2Cl_2$ (Pd_3); and (1,5-cyclooctadiene)$_2Rh_2$(2-allylphenolate)$_2$ (Rh_1).

0006-291X/82/010121-05$01.00/0

Materials and Methods

Albino Wistar rats weighing 200 ± 15 g were used throughout the experiments. Macrophages were obtained by collecting the peritoneal exudate (about 70% of macrophages) three days after a peptone solution was injected ip. The cells were washed three times with sterile isotonic saline solution by centrifugation at 800 rpm and suspended in TC medium (DIFCO) obtaining $3 \cdot 10^6$ cells cm^{-3}. The chemotaxis was evaluated using the Boyden chamber technique |6| as modified by Jungi |7|. The complexes were prepared as reported elsewhere (reference |4| for Pt_{10}; |8| for Pt_{11} and Pt_{13}; |9| for Pt_{12} and Pd_3; |10| for Rh_1).

10^{-2} or 10^{-3} M solutions of the complexes in dimethylsulphoxide (DMSO) were prepared and a calculated volume of each of them was added to the macrophages culture (overall volume 5 cm^3) in order to obtain a metal concentration ranging from 1.5 to 34 µg of metal per cm^3. The effective concentration was evaluated by atomic flame spectroscopy. The concentration of DMSO in the final medium was in any case lower than 2% v/v in order to avoid the solvent influence on the chemotaxis of the macrophages. The macrophages were incubated at the reported temperature for 30 min and then the supernatant liquid was discharged,the cells washed with an isotonic solution and the chemotaxis evluated at 37°C in any case. The viability of the cells was controlled using Trypan Blue in order to exclude that the diminished chemotactic response could be due to a cell damage.Using a concentration higher than 45 µg cm^{-3} of each of the drugs caused an evident cytoplasm disease. The LD_{50} for anyone of the complexes was 100 ± 5 µg cm^{-3}.

Discussion

The results obtained show that all the complexes used in this work inhibit the macrophages chemotaxis at a different extent dependent on the nature of the drug (Table 1). Moreover, on our opinion, it is noteworthy that,as the curves reported in Fig.1 show, the incubation of the macrophages with the various drugs at 0 °C,produces an evident and irreversible chemotactic defect also at concentrations of the drug as low as 3.4 µg cm^{-3} and for short time of contact.

In general, the biological activity of the transition metal complexes is related to the interaction with the nucleus and with nuclear DNA. An Interaction of this kind would affect,for sure,the macrophages chemotaxis. But the complexes would enter the macrophages essentially by a phagocytosis process and it is known that this process is annihilated at 0 °C. For that,one must expect that the concentration of the metal inside the cell should decrease as the incubation temperature approaches 0 °C and, however, at this temperature the chemotactic defect caused by the interaction of the drug with the nucleus should be negligible. Conversely,our results show that at 0 °C is observed a significant effect also at low concentration of the drug.

This low temperature effect is ascribed to the interaction of the complexes with the membrane of the cells. Such a interaction is purely chemical in character: it would depend on the nature of the complexes and on their concentration in the medium,but less effectively on the temperature change and on the time of contact,at

TABLE I. Depressive influence of the complexes on the Macrophages Chemotaxis.+

Compound	R_M (mean deviation)
CIS	0.55 (0.03)
Pt_{10}	0.036 (0.002)
Pt_{11}	0.40 (0.04)
Pt_{12}	0.062 (0.001)
Pt_{13}	0.16 (0.01)
Pd_3	0.38 (0.02)
Rh_1	0.35 (0.03)

+ The concentration of each drug was 30 μg of metal cm^{-3}

least considering the interval of several minutes |11| and the range of temperature explored in this work. This hypothesis is confirmed by the fact that,increasing the temperature,a relevant decrease of the chemotactic response is observed up to 20 °C, while there is no further variation in the interval 20 - 37 °C. This behaviour can be explained considering that at 20 °C the phagocytosis process is fully restored and,thus,the complexes can enter the cell and interact with the DNA and produce a further depressive effect on the chemotaxis.

In summary,we conclude that the overall chemotactic defect can be caused both from the interaction of the drugs with the membrane and with the nucleus of the cells and that the investigation of the dependence on the temperature of incubation is an useful tool that permits a rough evaluation of the relative importance of the two kinds of interaction.

When the influence of the concentration of the metal is considered,it can be point ed out that,as an average,a tenfold increase of the concentration of the metal produces a two- three-fold decrease of the chemotactic response.

Moreover,it is worth note that the various complexes used in this work influence at a different extent the chemotaxis of the macrophages changing the temperature. For example Pt_{10} (3.4 μg cm^{-3}) presents almost the same activity than Pt_{11} and Pt_{13} at the same concentration at 0 °C,but is much more active at 37 °C. This different behaviour can be related to the different stucture of the complexes and to their different ability to bind to membrane and DNA.

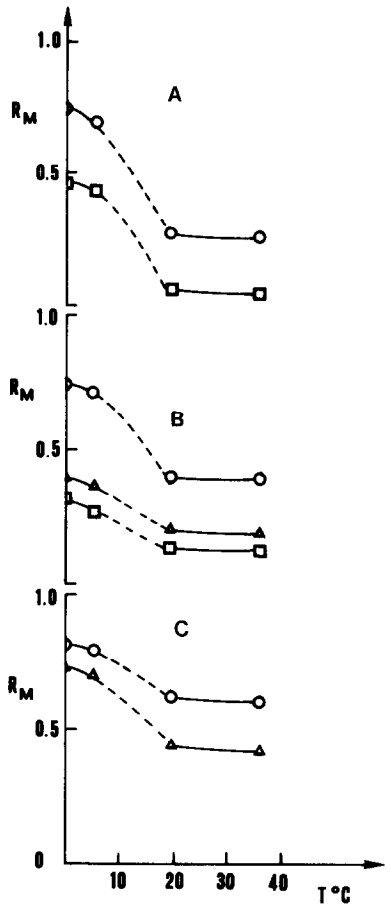

Figure 1

$R_M = \frac{\text{Number of macrophages migrated in the presence of the drug}}{\text{Number of macrophages migrated in absence of the drug}}$

Curves A refer to Pt_{10}, curves B to Pt_{13} and curves C to Pt_{11}.
The concentration of the metal for each drug was: O 3.4 μg cm^{-3} ; Δ 17 μg cm^{-3} ; □ 34.1 μg cm^{-3}. Each value reported is the mean of three experiments and the error ranges from 2 to 4 %.

Our view of the interaction of the complexes with the membrane of the macrophages almost agree with the fact that Pt(II) acts as a depolymerization agent on microtubules and filaments and causes loss of Ca^{2+}. These effects could be responsible of the reduced viability of the cells.

Binet [12] and Soodhi [13] have found other evidences for such interaction of Pt(II with the membrane.

Studies are in progress in order to determine the distribution of the metal between the membrane and the nucleus of the macrophages in dependence of the temperatur of incubation.

References

1. Fumarulo,R.,Giordano,D.,Riccardi,S.,Aresta,M., (1980) Proc.Soc.Exp.Biol.Med. 164, 164-166.
2. Fahey,J.L.,Brosman,S.,Ossorio,R.C.,O'Toole,C.,Zighdboim,J., (1976) Ann.Intern. Med. 84,454-465.
3. Hibbs,J.B.,Lambert,L.H.Jr,Remington,G.S.,(1972) Science 177,998-1003.
4. Aresta,M.,De Fazio,M.,Ingrosso,A.,Giordano,D.,(1980) J.Inorg.Nucl.Chem. 43,1415-1417.
5. Aresta,M.,Giordano,D.,Ingrosso,A.,Riccardi,S.,Sinigaglia,E.,(1979) Chem.Biol. Interac. 28,61-70.
6. Boyden,S.V.,(1962) J.Exp.Med.,115,453-466.
7. Jungi,T.W.,(1975) Int.Arch.Allergy Appl.Immun.48,341-352.
8. Aresta.M.,Greco,R.,(1979) Synt.Reac.Inorg.Met.-Org.Chem. 9,377-390.
9. Aresta,M.,De Fazio,M.,Ingrosso,A.,Bruno,P.,(1979) J.Inorg.Nucl.Chem. 41,1801-1804
10. Aresta,M.,Nyholm,R.S., (1975) J.Organometall.Chem. 56,395-403.
11. We have already shown (reference 1) that the time of incubation does not affect the chemotactic defect caused by CISPLATIN in the range 5 - 30 min.
12. Binet,A.,Volfin,P., (1977) Biochim.Biophys.Acta 461,182-187.
13. Soodhi,A., (1976) Indian J. Exp.Biol.,14,383-390.