Vol. 90, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 12, 1979 Pages 209-213

CORRELATION BETWEEN THE TOXICITY OF PLATINUM DRUGS TO L1210 LEUKAEMIA CELLS AND THEIR MUTAGENIC PROPERTIES

P. LECOINTE, J.P. MACQUET and J.L. BUTOUR

Laboratoire de Pharmacologie et de Toxicologie fondamentales
du CNRS
205, route de Narbonne, 31078 TOULOUSE Cedex, FRANCE

Received June 12,1979

There is a good correlation between the growth inhibitory properties of a series of Pt(II) derivatives on cultured L1210 mouse leukaemia cells and their efficiency of reversion to *his*$^+$ of *S. typhimurium* TA100, or with their forward mutagenic effects on prophage λ. Reactivity towards DNA may thus explain the antitumor properties of these drugs.

The antitumor derivatives of platinum(II) are square planar coordination compounds which depend for their therapeutic efficiency on the presence of two exchangeable ligands, oriented in *cis* position around the central Pt atom (bond angle of *ca.* 90°) (1,2). Their bifunctional reactivity towards nucleic acids leads to the formation of adducts between the *cis* derivatives and guanine, such as intrastrand chelates with N7 and O6 (3). As they are strong electrophilic reagents, their reactivity extends to other macromolecules. The biological target of their effects remains therefore to be identified. Covalent binding to DNA explains their lethal effects on bacteria (increased sensitivity of *uvr* mutants over wild types) (4). Excision-repair deficient human fibroblasts are also more sensitive to these drugs (5). For a few derivatives, a relation holds between their efficiency of reversion of a nonsense mutant of *E. coli* and the decrease in tumor (Sarcoma 180) weight (4).

Abbreviations: en : ethylenediamine
dien : diethylenetriamine
ox : oxalato

0006-291X/79/170209-05$01.00/0

We demonstrate in this paper that the sensitivity of a tumorigenic mouse cell line to Pt compounds depends on the same structural features as those required for mutagenesis in two prokaryotic test systems. We interpret this correlation as evidence that DNA is the target for the toxic effects of these drugs on L1210 cells and hence, probably, their antitumor properties.

Methods : L1210 cells were obtained from the ascitic fluid of female DBA/2 mice, and grown in RPMI medium supplemented with 20 % heat inactivated horse serum, 2 mM L-glutamine, 200 U/ml penicillin and 50 µg/ml streptomycin. Every 2 or 3 days we divided the cultures into fresh medium to 1-2 x 10^5 cells/ml. Incubation was done without shaking in 20 ml medium (100 ml bottles) in a 5 % CO_2 atmosphere at 37°. Duplicate determinations of the number of viable cells (cells able to exclude Trypan blue) were performed each day for 2 days, during which the growth remained exponential. The ID_{50}s (concentration of drug that decreases by 50 % the growth rate of the cells) were determined by adjusting the concentrations of drug (0.05-2000 µg/ml) in 20 ml of culture at 10^5 cells/ml. After 1 and 2 days of growth in drug-containing medium, the inhibition was calculated from the slope of the growth line.

The determination of the mutagenic potency of the Pt drugs was performed first on the *Salmonella* strain TA100 (6) as described by Ames (7). Methylmethanesulfonate was used as positive control (0.5 revertant per nanomole). Duplicate experiments differed usually by less than 20 %, and the results were taken from the linear part of the dose-response curve.

The bacteriophage λ mutagenesis assay (8) measures the increase in the frequency of forward mutation to virulence of λGY14[1], a *b2*$^+$ recombinant of λ11 *b2v2v3* with phage 434. We use in this test the lysogenic *E. coli* GY4854 *envA, uvrB34*, λGY14, and the indicator strain GY3646. The latter bears a wild type λ and is normally immune to superinfection. Nevertheless, when a third operator mutation (*v1*) has occurred in λGY14, it is able to infect the lysogenic indicator strain, and give clear lysis plaques on GY3646.

The lysogenic induction assay (8,9) was performed on GY5027 (*envA, uvrB,* λ) with indicator strain GY4015. In the two last methods, we used mitomycin C as positive control (giving respectively a 5 x 10^4 fold increase in the yield of virulent GY14 mutants over the background (*ca.* 10^{-7}) rate , and *ca.* 60 % of infective centers from the number of GY5027 lysogenic bacteria plated, at doses of 0.1 and 1 µg per plate, respectively. In both viral assays, the number of bacteria per plate was determined by plating suitable dilutions, and control experiments were run to measure the background of resp. spontaneous mutation frequency or lysogenic induction.

Results :

The efficiency of reversion of the point mutation *his*G46 in TA100 by the Pt(II) drugs has been plotted in fig. 1a against the 50 % inhibitory concentration for L1210 cells. A very good correlation appears between these two effects (least squares adjustement, $r = .902$, $p < 0.001$). A straight line can also be drawn for the λ mutagenesis assay but the limited number of mutagenic derivatives available prevented us from making the same calculations.

[1] λGY14 replaces λGY13 used in ref. (8). DEVORET, pers. communication and gift.

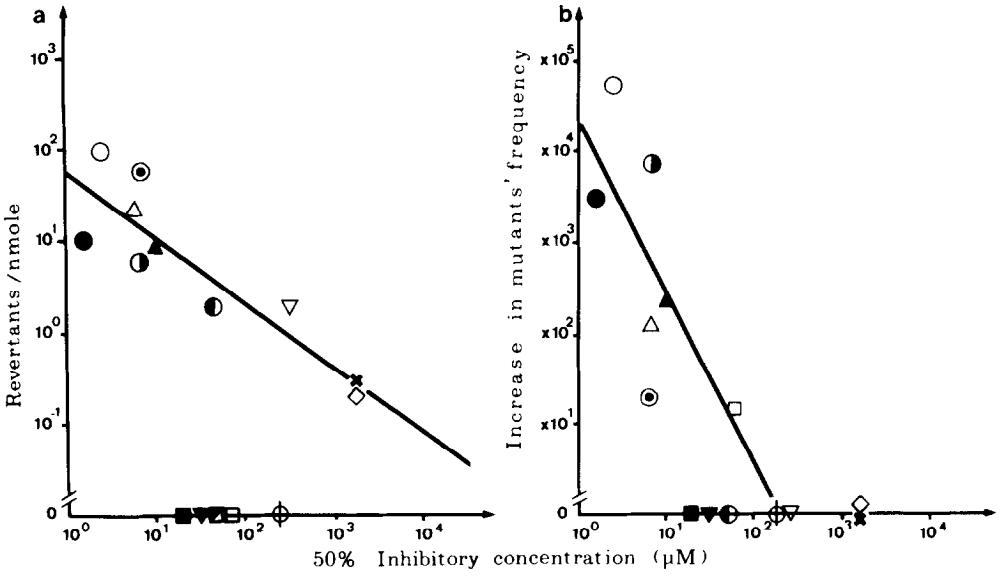

Fig. 1a (left) : Mutagenic properties of platinum compounds in the *Salmonella* test *vs.* ID_{50}. ○ : *cis*-$Pt(NH_3)_2Cl_2$; △ : *cis*-Pt(en)Cl_2 ;
▽ : [Pt(dien)Cl]Cl ; ◇ : $[Pt(NH_3)_4]Cl_2$;
● : *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$; ▲ : *cis*-$[Pt(en)(H_2O)_2](NO_3)_2$;
◑ : $K[Pt(NH_3)Cl_3]$; ◐ : $K_2[PtCl_4]$; x : $[Pt(en)(NH_3)_2]Cl_2$;
⊙ : *cis*-$Pt(NH_3)_2I_2$; ■ : *trans*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$;
▼ : $[Pt(dien)(H_2O)](NO_3)_2$; □ : *trans*-$Pt(NH_3)_2Cl_2$;
Φ : *trans*-$Pt(NH_3)_2I_2$; ◪ : *trans*-$[Pt(NH_3)_2(ox)_2]K_2$

Fig. 1b (right) : Mutagenic properties in the λ test *vs.* ID_{50}

Some compounds as [Pt(dien)Cl]Cl or $K_2[PtCl_4]$ have no significant activity in the phage system, although they are definitely mutagenic in the *Salmonella* assay. As they have been shown to react with DNA *in vitro* (10), we looked for a permeability artifact by determining the efficiency of λ induction, and found (Table I) that every derivative bearing at least one labile group (-Cl or $-H_2O$) was able to induce prophage λ . These results demonstrate that electrophilicity is not the only requirement for the expression of mutagenic and toxic effects, but that the action of Pt drugs requires some very specific structural features.

The finding that compounds like [Pt(dien)Cl]Cl or $K_2[PtCl_4]$ are λ inducers but not mutagens in the phage system has many implications. [Pt(dien)Cl]Cl can bind to the DNA of L1210 cells to a ratio of 1 Pt to 1000 base pairs without any effect on cell viability (11), suggesting that adduct formation with DNA has little effect on replication. The inducing, but not mutagenic

Table I

Inducing potency of Pt compounds

Compound	% Induction
cis-$Pt(NH_3)_2Cl_2$	90
cis-$[Pt(NH_3)_2(H_2O)_2]^{++}$	100
cis-$Pt(en)Cl_2$	75
cis-$[Pt(en)(H_2O)_2]^{++}$	80
cis-$Pt(NH_3)_2I_2$	10
trans-$Pt(NH_3)_2I_2$	5
trans-$Pt(NH_3)_2Cl_2$	15
trans-$[Pt(NH_3)_2(H_2O)_2]^{++}$	20
trans-$[Pt(NH_3)_2(ox)_2]K_2$	25
$K_2[PtCl_4]$	40
$[Pt(NH_3)Cl_3]^-$	100
$[Pt(NH_3)_3Cl]^+$	20
$[Pt(dien)Cl]^+$	75
$[Pt(dien)(H_2O)]^{++}$	toxic
$[Pt(NH_3)_4]^{++}$	0
$[Pt(en)(NH_3)_2]^{++}$	0

The inducing potency is defined as the percentage of induced lysogens (infective centers) after treatment with Pt inducers, calculated from the number of plated bacteria. The background of spontaneous induction was about 1-5 %.

properties of this class of reagents can be explained in at least two ways : direct inactivation of repressor (9) or a transient inhibition of the progress of the replication fork, which could suffice to induce the "SOS functions", but would leave unaltered the template capacity of DNA. Minor lesions like these require the mutator effect of pKM101 in Ames' strains to be detected as mutagenic events.

We conclude that since the mutagenic and toxic effects on L1210 cells are well correlated, DNA is most probably the primary target of the antitumor effect, although the basis of the specificity towards cancer cells, as can

be observed *in vivo*, remains to be established. As expected from the mutagenicity data, the carcinogenic properties of Pt drugs have been recently demonstrated (12). The present work makes it doubtful whether it is possible to abolish this secondary effect while keeping the antitumor property.

Acknowledgments : We are grateful to Drs. Devoret and Moreau for introducing us to the λ assays, and M. Germanier and A.M. Mazard for excellent technical assistance.

REFERENCES

1. Rosenberg, B., Van Camp, L., Trosko, J.E. and Mansour, V.H. (1969) Nature 222, 385-386.
2. Connors, T.A. and Roberts, J.J. eds (1974) Platinum Coordination Complexes in Chemotherapy, Springer-Verlag, Berlin.
3. Macquet, J.P. and Theophanides, T. (1975) Bioinorg. Chem. 5, 59-66.
   Millard, M.M., Macquet, J.P. and Theophanides, T. (1975) Biochim. Biophys. Acta 402, 166-170.
4. Beck, D.J. and Brubaker, R.R. (1973) J. Bacteriol. 116, 1247-1252.
5. Fraval, H.N.A., Rawlings, C.J. and Roberts, J.J. (1978) Mutation Res. 51, 121-132.
6. Lecointe, P., Macquet, J.P., Butour, J.L. and Paoletti, Cl. (1977) Mutation Res. 48, 139-144.
7. Ames, B.N., MacCann, J. and Yamasaki, E. (1975) Mutation Res. 31, 347-363.
8. Moreau, P., Bailone, A. and Devoret, R. (1976) Proc. Nat. Acad. Sci. USA 73, 3700-3704.
   Moreau, P. and Devoret, R. (1977) Origins of Human Cancer, pp. 1451-1452, book C, Hiatt, H.A., Watson, J.D. and Winsten, J.A. eds., Cold Spring Harbor.
   Devoret, R., Anderson, A. and Moreau, P. to be published.
9. Reslova, S. (1971/1972) Chem.-Biol. Interactions 4, 66-70.
10. Butour, J.L. and Macquet, J.P. (1977) Eur. J. Biochem. 78, 455-463.
11. Butour, J.L. and Macquet, J.P. (1978) Biochimie 60, 901-914.
12. Leopold, W.R., Miller, E.C. and Miller, J.A. (1979) Cancer Res. 39, 913-918.