Vol. 112, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 29, 1983 Pages 555-563

CIS-$Pt(NH_3)_2Cl_2$ AND TRANS-$Pt(NH_3)_2Cl_2$ INHIBIT DNA SYNTHESIS IN CULTURED L1210 LEUKEMIA CELLS

B. SALLES, J.L. BUTOUR, C. LESCA AND J.P. MACQUET

LABORATOIRE DE PHARMACOLOGIE ET DE TOXICOLOGIE FONDAMENTALES DU C.N.R.S.
205 route de Narbonne, 31400 TOULOUSE, France

Received February 22, 1983

SUMMARY : A comparison of the inhibition of DNA synthesis by the two geometrical bidentate isomers cis- and trans-$Pt(NH_3)_2Cl_2$ and by the monodentate [Pt(dien)Cl]Cl in a model used for screening potential antitumor compounds, the L1210 leukemia cells, is presented. The efficacy of penetration after a 2 hours Pt treatment is in the order trans (8) > cis (1) ∿ dien (0.7). DNA replication is reduced to 50 % of the control when 1.8×10^{-4}, 2.4×10^{-4} and 80×10^{-4} Pt atoms were bound per nucleotide for cis, trans and dien derivatives, respectively. If we admit that DNA is the pharmacological target of Pt antitumor compounds, these results suggest that a quantitative inhibition of DNA synthesis is certainly not correlated with antitumor activity.

The discovery of cis-$Pt(NH_3)_2Cl_2$ (abbreviated cis-DDP) as an anti-neoplastic compound toward experimental tumor systems by Rosenberg et al. (1), has led its extensive use in clinical cancer chemotherapy (2). Cis-DDP binds covalently to nucleophilic macromolecules like DNA, RNA and proteins (3,4) and inhibits DNA replication in a greater extent than transcription or protein synthesis (5). Fixation of cis-DDP on DNA was proposed to account for its cytotoxicity and correlated with antitumor activity (for a review see ref. 6). On the other hand, the trans isomer did not reveal any significant antitumoral properties (7,8) although it binds to DNA in vivo (9), and is far less cytotoxic (3,8,10-12) and mutagenic than the cis isomer (10,12-16).

ABBREVIATIONS : PBS = phosphate buffer saline ; SDS = sodium dodecyl sulfate ; TCA = trichloracetic acid ; dien = diethylenetriamine ; ID_{50} = median inhibitory dose, a drug concentration that decreases the growth rate of L1210 cells to 50 % of that of control ; r_b = number of Pt atoms bound per nucleotide.

0006-291X/83 $1.50

This study reports potential effects of cis-DDP, trans-DDP and [Pt(dien)Cl]Cl in the process of DNA replication in L1210 cells for equivalent r_b values. The L1210 leukemia cell line was selected because it is currently used for screening antitumoral drugs (17) and these three platinum(II) compounds exhibited different cytotoxicities and antitumor activities in this model (8). Moreover, these compounds are representative of the three different classes of DNA secondary structure destabilization for in vitro Pt-DNA interaction (18).

MATERIALS AND METHODS

Chemicals. The platinum(II) compounds were synthetized as previously reported (19). Platinum solutions, twice the final concentration, were freshly prepared in RPMI (Gibco) medium. [methyl-^{3}H]thymidine (20 Ci/mmole, specific activity) was obtained from the Commissariat à l'Energie Atomique, Saclay, (France).

Cells and Culture Medium. Mouse L1210 leukemia cells were grown as previously reported (8) and cell counting was carried out with a Coulter counter (ZBI).

Inhibition of DNA Synthesis. Cells in exponential phase of growth, were pooled by centrifugation and resuspended in serum free medium at 1.2-1.6 x 10^6 cells/ml. 0.5 ml of cell suspension were added to 0.5 ml of RPMI medium containing increased platinum concentration in polystyrene tubes and kept at 37°C under gentle stirring for 2 hours. Cells were then centrifuged (1000 g, 7 min, 20°C) and cultivated in 1 ml radioactive medium (0.5 µCi/ml of [methyl-^{3}H]thymidine). Following the labelling period, 3 ml of cold PBS was added in each tube. Cells were centrifuged, washed in 3 ml cold PBS and after centrifugation (1000 g, 7 min, 4°C) the pellet was lysed in 1 ml Tris 50 mM, pH 8, 0.5 % SDS solution and 0.1 ml calf thymus DNA (2 mg/ml, Sigma), 1.1 ml cold TCA 10 % were added in each tube. The tubes were kept in ice for 30 minutes and the amount of radioactive material in the acid insoluble portion was determined by filter (GF/C Whatman) binding assay. The amount of radioactivity was measured in an Intertechnique SL30 liquid scintillation spectrometer with a Beckman Ready Solv EP scintillation fluid.

DNA Isolation. A similar experimental procedure was used with 20 ml of cell suspension in glass bottles instead of 1 ml. L1210 cells were centrifuged (1100 g, 7 min, 4°C) and washed three times with 20 ml of 150 mM NaCl. After the last washing (no platinum could be detected) the cells were resuspended in 3ml of TNE buffer (Tris 50 mM, pH 8.0, NaCl 100 mM, EDTA 5 mM), and sonicated with a Branson B12 Sonifier at 4°C during three periods of 30 seconds. An aliquot was kept to quantify the platinum in the whole cells. DNA was extracted two times with phenol saturated with TNE buffer and dialyzed against 10 mM $NaClO_4$. The DNA concentration was determined by absorbance at 260 nm with a Zeiss PMQII spectrophotometer assuming that 10 µg/ml corresponds to $A_{260} = 0.210$.

Platinum Determination. The experimental procedure has been reported (19).

RESULTS

Fig. 1 shows the kinetics of [methyl-^{3}H]thymidine incorporation after a 2 hours exposure to cis-DDP, trans-DDP and [Pt(dien)Cl]Cl. The rate of

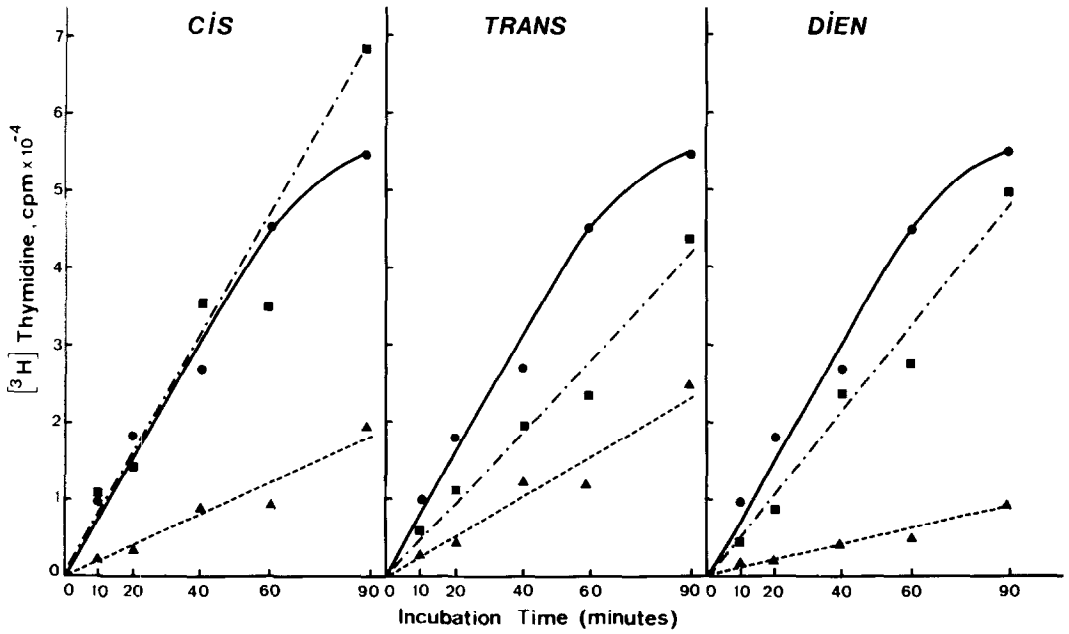

Figure 1. Kinetics of [methyl-^{3}H]thymidine incorporation after a 2 hours platinum treatment ; cis : (■) 15 µg/ml ; (▲) 150 µg/ml ; (●) control ; trans : (■) 15 µg/ml ; (▲) 75 µg/ml ; (●) control ; dien : (■) 1.5 mg/ml ; (▲) 3 mg/ml ; (●) control. Experiment in duplicate.

[methyl-^{3}H]thymidine incorporation was linear up to 1 hour in the control and in the treated cells. When cells were pretreated with 15 µg/ml of cis- or trans-DDP, a decrease in the [methyl-^{3}H]thymidine incorporation was observed only in the case of the trans isomer. At the same platinum(II) concentration (Fig. 1) trans-PDD induced a decrease of [methyl-^{3}H]thymidine incorporation two times greater than cis-PDD. A 30 fold higher [Pt(dien)Cl]Cl concentration was necessary to observe an inhibitory effect similar to that of trans-DDP. Different covalent binding of the three platinum(II) compounds in L1210 cells are represented in Fig. 2. The platinum fixation curves were linear until a concentration of 150 µg/ml. For higher concentrations (300 µg/ml) cis-DDP showed a "two-hit" response curve, however trans-DDP could not be tested at that level due to its lower solubility. The efficacy of penetration was found eight times higher for the trans than for the cis or the dien derivatives at a concentration of 150 µg/ml (Table I). The r_b values were determined in order to circumvent the different uptake and reactivity of the three Pt(II) compounds. Since it appeared that the platinum(II) compounds used interfere

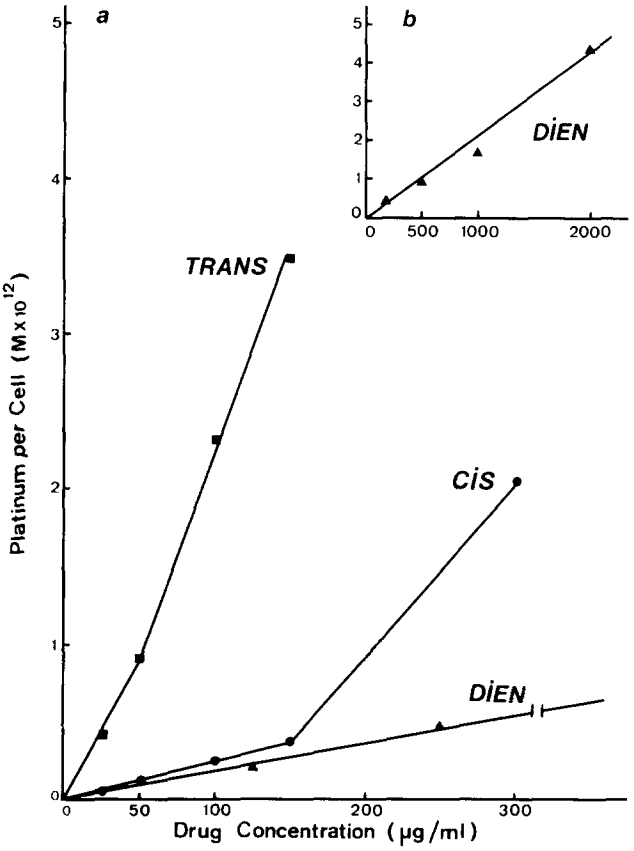

Figure 2. Platinum concentration covalently bound at the cellular level after a two hours exposure versus drug concentration (insert : higher levels of dien derivative). Experiment in triplicate.

with the rate of DNA synthesis, a 1 hour labelling period was selected to investigate the dose-effect relationships. Fig. 3 shows the inhibitory effect of each Pt(II) compound on DNA synthesis versus their fixation on DNA. The r_b values which correspond to a 50 % decrease of DNA synthesis are 1.8 x 10^{-4}, 2.4 x 10^{-4} and 80 x 10^{-4} respectively for cis-DDP, trans-DDP and [Pt(dien)Cl]Cl. Trans-DDP was overconcentrated in the whole cell (8 times more than cis-DDP) but its fixation on DNA was less than two fold higher than that of cis-DDP. This discrepancy could be due to the higher reactivity of trans-DDP which may bind to nucleophilic centers before it penetrates into the nucleus. The cis-DDP lesions on DNA at that level of binding are then less than two times more efficient to inhibit DNA replication than the trans-DDP lesions and 45 times more efficient than the [Pt(dien)Cl]Cl

TABLE I

CYTOTOXICITY, PENETRATION, DNA BINDING AND RELATIVE BIOLOGICAL ACTIVITIES FOR THE THREE Pt(II) COMPOUNDS IN LEUKEMIA L1210 CELLS.

Biological Activity and Pt Binding	cis-$Pt(NH_3)_2Cl_2$ antitumoral	trans-$Pt(NH_3)_2Cl_2$ non-antitumoral	[Pt(dien)Cl]Cl non-antitumoral	References
ID_{50} (μM)	2.3	67	271	8
Pt/Cell($\times 10^{16}$g) corresponding to ID_{50}	< 2(25)[a]	200	110	this work
r_b corresponding to ID_{50}	$< 5 \times 10^{-6}$ [b]	4×10^{-5}	5×10^{-4}	this work
r_b corresponding to 50 % of inhibition of DNA synthesis	1.8×10^{-4}	2.4×10^{-4}	80×10^{-4}	this work
Inhibition of DNA replication (L1210)[c]	1-2	1	0.03	this work
Toxicity[c] : - Hela	5	1	ND[d]	3
- CHO	9	1	ND	12
- L1210 (ID_{50})	> 10	1	0.1	this work
Mutagenicity (CHO)[c]	750	1	ND	12

[a]This value in parentheses corresponds to a cis-DDP concentration of 67 μM, i.e., the ID_{50} value for trans-DDP ; [b]Not detected (detection limit = 1 Pt atom/200 000 nucleotides, [DNA] = 1 mg/ml) ; [c]These relative activities are related to trans-$Pt(NH_3)_2Cl_2$; [d]Not done.

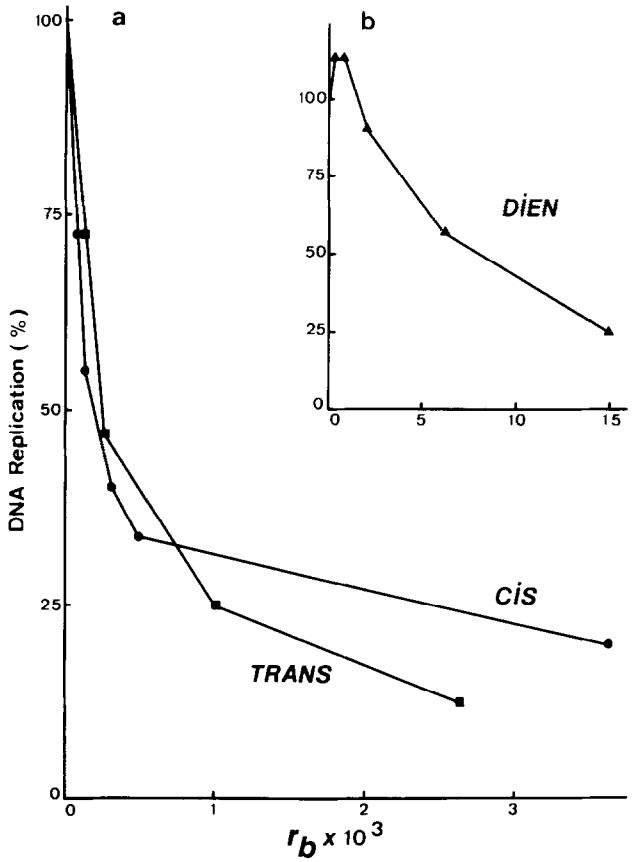

Figure 3. Correlation between the inhibition of DNA replication and the number of platinum atoms fixed per nucleotide (r_b) (insert: inhibition of DNA replication for the dien derivative). Experiment in triplicate.

lesions. For both isomers, an almost quantitative inhibition of DNA synthesis (70-80 %) was observed for r_b values close to 4-6 x 10^{-4}, which means that the cis- and trans-Pt lesions at that level, inhibit almost equally the DNA synthesis. [Pt(dien)Cl]Cl is far less efficient under these conditions (r_b = 150 x 10^{-4} for 75 % inhibition).

DISCUSSION

The binding of the two stereoisomers cis- and trans-DDP at the cellular level is not specifically directed toward DNA. In Hela cells and in Chinese hamster V79-379A cells, Roberts et al. (3,4) reported also a binding on RNA and proteins. However, a number of biological and biochemical experiments (inhibition of DNA synthesis, different repair ability, mutagenicity,

induction of prophage λ, induction of recA protein) suggest that DNA could be the pharmacological target responsible for the activity of cis-DDP (6,20). There is no doubt that DNA is a target for cis-DDP, since platinum was quantified on the macromolecule in vivo in L1210 cells (9) grafted in mice and also in cultured cells like Hela (3), CHO cells (12) and E. coli (11). However, a clear-cut correlation between antitumor activity of these compounds, including active and inactive compounds and a biological parameter has never been published to our knowledge. The two non-antitumoral compounds trans-DDP and [Pt(dien)Cl]Cl also bind to DNA in vitro (21) and in vivo (9). Their biological efficacy per platinum bound on DNA is significantly lower than for the cis isomer (Table I). Based on the results of Harder and Rosenberg (22) it was generaly accepted that the selective and persistent inhibition of DNA synthesis observed for the cis-Pt compounds, might be correlated with antitumor activity. To prove such a correlation, experiments must be conducted with the same cells in vitro (inhibition of DNA synthesis) and in vivo (antitumor activity). The experiments presented in this study do not confirm the results of Harder and Rosenberg even if cis-DDP is less than two times more efficient than the trans-isomer to inhibit DNA synthesis per Pt bound on DNA. Trans-DDP was also found to inhibit DNA replication in E. coli (11,23) and in CHO cells (24). A recent study (25) confirmed the fact that cis-DDP and $K_2[PtCl_4]$ (non-antitumoral) inhibit DNA synthesis to the same extent in two tumor cell lines. However, none of these studies have correlated the inhibition of DNA synthesis with the antitumoral properties of the Pt compounds and with the amount of covalently bound Pt atoms on DNA.

At identical ID_{50} values, Pt bound on DNA as the cis form is at least 10 times more cytotoxic than in the trans form and 100 times than in the dien form. If the fixation of Pt on DNA is responsible for the cytotoxicity of these compounds, the very different growth inhibition rates reported in Table I associated with different Pt-DNA binding might be correlated with different Pt-DNA adducts which are under study in our laboratory (9).No relationship seems to exist between cytotoxicity and inhibition of DNA synthesis (Table I)

since cis-DDP is 30 times more cytotoxic than trans-DDP and both compounds inhibit DNA synthesis to the same extent. However, cytotoxicity has been correlated with antitumor activity in a series of Pt compounds (8). It must be noted that for a concentration corresponding to the ID_{50} value, there is no inhibition of DNA synthesis with cis-DDP whereas a 25 % inhibition is found with trans-DDP and no inhibition with the dien derivative. This result can be compared with those obtained in Physarum polycephalum where giant polyploid nuclei were observed at non-toxic concentrations only with antitumoral Pt compounds without any quantitative effect on DNA replication (26). In conclusion, even if DNA is a target for antitumor and non-antitumor Pt compounds, the biological parameter which can be correlated with the antitumor properties of these compounds is not a quantitative inhibition of DNA synthesis.

ACKNOWLEDGEMENTS. The financial support of F.E.G.E.F.L.U.C. is gratefully acknowledged. B.S. is Assistant at the University Paul Sabatier (Faculté de Pharmacie), Toulouse. We would like to thank Dr. R. Alazard for critical reading of the paper and Dr. S. Cros for facilities in L1210 cells handling.

REFERENCES

1. Rosenberg, B., VanCamp, L., Trosko, J.E., and Mansour, V.H. (1969) Nature (London) 222, 385-386.
2. Einhorn, L.H. (1981) Cancer Res. 41, 3275-3280.
3. Pascoe, J.M., and Roberts, J.J. (1974) Biochem. Pharmacol. 23, 1345-1357.
4. Van den Berg, H.W., and Roberts, J.J. (1976) Chem.-Biol. Interactions 12, 375-390.
5. Howle, J.A., and Gale, G.R. (1970) Biochem. Pharmacol. 19, 2757-2762.
6. Roberts, J.J., and Thomson, A.J. (1979) Progress in Nucleic Acid Research and Molecular Biology (Cohn, W.E., Ed.) Vol. 22, pp. 71-133, Academic Press, New York.
7. Cleare, M.J., and Hoeschele, J.D. (1973) Bioinorg. Chem. 2, 187-210.
8. Macquet, J.P., and Butour, J.L. (1983) J. Natl. Cancer Inst. in press.
9. Macquet, J.P., Butour, J.L., and Johnson, N.P. (1983) Amer. Chem. Soc. Symposium Series, in press.
10. Zwelling, L.A., Matthews, O.B., Sharkey, N.A., Anderson, T., and Kohn, K. (1979) Mutation Res. 67, 271-280.
11. Alazard, R., Germanier, M., and Johnson, N.P. (1982) Mutation Res. 93, 327-337.
12. Johnson, N.P., Hoeschele, J.D., Rahn, R.O., O'Neill, J.P., and Hsie, A.W. (1980) Cancer Res. 40, 1463-1468.
13. Beck, D.J., and Brubaker, R.R. (1975) Mutation Res. 27, 181-189.
14. Lecointe, P., Macquet, J.P., Butour, J.L., and Paoletti, C. (1977) Mutation Res. 48, 139-144.
15. Beck, D.J., and Fisch, J.E.(1980) Mutation Res. 77, 45-54.
16. Leopold, W.R., Batzinger, R.P., Miller, E.C., Miller, J.A., and Earhart, R.H. (1981) Cancer Res. 41, 4368-4377.

17. Geran, R.I., Greenberg, N.H., Macdonald, M.M., Schumacher, A.M., and Abbott, B.J. (1972) Cancer Chemother. Rep. 3, 1-103.
18. Macquet, J.P., and Butour, J.L. (1978) Biochimie 60, 901-914.
19. Butour, J.L., and Macquet, J.P.(1981) Biochim. Biophys. Acta 653, 305-315.
20. Salles, B., and Lesca, C. (1982) Biochem. Biophys. Res. Commun. 105, 202-208
21. Macquet, J.P., and Theophanides, T. (1975) Biopolymers 14, 781-799.
22. Harder, H.C., and Rosenberg, B. (1970) Int. J. Cancer 6, 207-216.
23. Kohl, H.H., Haghighi, S., and McAuliffe, C.A. (1980) Chem.-Biol. Interactions 29, 327-333.
24. Plooy, A.C., and Lohman, P.H.M. (1980) Toxicology 17, 169-176.
25. Hurwitz, E., Kashi, R., and Wilchek, M. (1982) J. Natl. Cancer Inst. 69, 47-51.
26. Wright, M., Lacorre-Arescaldino, I., Macquet, J.P., and Daffé, M., submitted for publication.