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INTERACTION OF *CIS*-DIAMMINEDICHLOROPLATINUM (II) WITH SENSITIVE AND RESISTANT L1210 CELL LINES

DRUG BINDING TO NUCLEI AND DNA

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Abstract—We used in parallel, to study the kinetics of *cis*-DDP cellular binding and distribution, a cL cell culture line established from L1210 murine leukemia ascites and its cLP derivative which acquired a 30-fold (ID_{50}) resistance to *cis*-diamminedichloroplatinum(II). Cell cultures were incubated with 0.9 μg/ml (3 μM) of the drug and after various incubation times up to 24 hr, the amount of platinum associated to whole cells, to isolated nuclei and to purified DNA was determined using atomic absorption spectrophotometry.

For the first hours of incubation no significant difference in the rate of platinum association was observed between the two cell lines. After the first hours of incubation the amount of platinum associated to whole cells and to isolated nuclei was significantly higher in the drug sensitive cells. However, the rates of platinum association to the respective DNAs were quite similar in the two cell lines.

Our study failed to demonstrate any significant quantitative modification of the overall drug–DNA association between the resistant and sensitive cell lines.

Cis-diamminedichloroplatinum (II), *cis*-DDP† or cisplatin is the first inorganic compound to have been used as an efficient antitumor agent in human therapy [1].

The molecule is highly electrophilic and reacts in cells with many different types of molecules bearing nucleophilic groups, such as DNA, RNA and proteins [2, 3]. Much evidence from *in vitro* and *in vivo* systems suggests that the DNA might be a critical intracellular target of the platinum (II) drugs [4]. Furthermore, the binding of the drug to DNA is related to its cytotoxicity and antitumor activity [5–7]. Biological inactivation of platinum modified DNA has already been described [8, 9] and platinum mutagenicity to DNA has also been demonstrated [10].

Cis-DDP reacts bifunctionally with DNA to form both DNA-interstrand and DNA-intrastrand cross-links as well as DNA-protein cross-links [11–13] DNA-protein cross-links show little correlation with cytotoxicity and are considered to be innocuous lesions [14]. However, there is disagreement as to whether inter- or intrastrand cross-linking is most critical.

A murine leukemia L1210 cell culture subline (cLP) has been developed which is resistant (ID_{50}) to concentrations of *cis*-DDP 30-fold higher than its parental cell culture line (cL). Such material is useful to study a possible correlation between the acquired drug resistance and the mode of drug binding. Therefore, we studied the uptake and cellular distribution of the drug in the parent cL cell line and the *cis*-DDP-resistant cLP subline derived from the L1210 murine transplantable leukemia.

The present paper reports that under our incubation conditions and for periods of time shorter than 6 hr, the respective amounts of drug taken up by nuclei as well as the respective amounts of DNA-bound drug were similar. Moreover, under conditions where the amount of *cis*-DDP bound to nuclei was significantly higher in the sensitive line than in the resistant line, the amount of the DNA-bound drug was of the same order in those two cell lines.

MATERIALS AND METHODS

Cell lines

The cL drug-sensitive cell line and its *cis*-DDP-resistant derivative termed cLP were used in this study. Cell cultures were first initiated using L1210 transplantable leukemia cells maintained by serial passages in syngeneic DBA/2 mice, kindly provided by Dr Charles Gosse, Institute Gustave Roussy. Cultures were established as cell suspension in the RPMI 1640 medium supplemented with 10% fetal calf serum, 100 I.U./ml penicillin and 100 μg/ml streptomycin. After 2 months, the serum concentration was reduced to 8%. The resulting suspension cell culture line, designated cL, proliferated regularly with a population doubling time of 12–14 hr as determined from exponential growth curves.

After 2 months of growth in culture of the cL cells,

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† Abbreviations used: *cis*-DDP, *cis*-diamminedichloroplatinum (II); Tris, trie-(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethylsulfonylfluoride; RPMI, Roswell Park Memorial Institute; *r*, ratio of bound platinum per nucleotide of DNA.

the cLP subline was derived by exposure to gradually increasing concentrations of *cis*-DDP, starting with 24 hr of exposure to 0.3 μg/ml and achieving permanent exposure to 2 μg/ml within 7 months.

To assess the tumorigenic capacity of the original L1210 and the cultured cL and cLP cells, 10^5 cells of each of these three strains were inoculated intraperitonealy into age-matched female DBA/2 syngeneic mice. Each experimental group including at least 15 animals.

Cis-*DDP*

This drug was synthesized by Dr. J. P. Macquet (Laboratoire de Pharmacologie et Toxicologie Fondamentale du CNRS, Toulouse) according to the Dhara technique [15]. The stock solution of the chemical was prepared under sterile conditions at a concentration of 1.54 mg/ml (i.e. platinum concentration = 1 mg/ml) in isotonic sodium chloride (0.15 M). Purity and stability of the stock solution were tested with high performance liquid chromatography (Waters chromatograph equiped with a C18 μ-Bondapack column).

Cell treatments for drug binding studies

Cells (1.5×10^4 cells per ml) were first sseded in drug-free medium (RPMI 1640 medium supplemented with 6% fetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin) and after 3 days of log growth (6×10^5 cells per ml) they were incubated with 0.9 μg/ml (3 μM) of *cis*-DDP for 2, 4, 10 and 24 hr. For each time point, cells were sampled, counted and divided in three aliquots: "a" (125 ml, 2.4×10^5 cells/ml), used to measure the total amount of platinum bound to cells; "b" (125 ml, 2.4×10^5 cells/ml), used to isolate nuclei and to measure the amount of drug bound to nuclei; "c" (250 ml, 2.4×10^5 cells/ml), used to extract DNA and to measure the amount of drug bound to it.

Determination of the amount of platinum bound to cells

The cells from aliquot "a" were resuspended in fresh medium without serum (30 ml), homogenized and centrifuged at 2000 rpm for 10 min. This washing process was repeated four times (no platinum detected in the last washing medium). The last cellular pellet was homogenized in 0.2 ml of nitrous acid (13.7 N). The amount of platinum was then measured using an atomic absorption spectrophotometer (see below).

Nuclei isolation

The cellular pellet obtained from aliquot "b" thoroughly washed as above and centrifuged, was resuspended at 4° in ice-cold extraction buffer containing 10 mM Tris–HCl, pH 7.4, 50 mM NaCl, 25 mM KCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$ and PMSF at a final concentration of 0.1 mM. Nonidet P40 (Fluka Switzerland) was then added to reach a final concentration of 0.25% (v/v). After 15 min at 4° under slight agitation, the cellular suspension was centrifuged at 2000 rpm for 10 min. The nuclear pellet was resuspended in the extraction buffer and the quality of the nuclei preparation checked under light microscope which is determined by the absence of extranuclear material. The amount of associated platinum was measured using an atomic absorption spectrophotometer (see below).

DNA purification

Nuclear pellets from aliquot "c" were resuspended in 1.5 M NaCl, 1.0 mM EDTA pH 8.0 and incubated for 1 hr at 37° in the presence of 20 μg/ml of pancreatic ribonuclease (Boehringer Mannheim). Sodium dodecyl sulfate (0.5% final concentration) and proteinase K (Boehringer Mannheim at 50 μg/ml were then added and the incubation was further extended for 2 more hours. Solutions were then deproteinized with chloroform-isoamyl alcohol (24:1 v/v) and the DNA precipitated with two volumes of 99.85% ethanol, washed several times with 99.85% ethanol and dried. The amount of DNA was determined through UV absorption at 260 nm and the amount of bound platinum by the means of an atomic absorption spectrophotometer. Previously to this experiment, it has been checked that the purification process did not induce any loss of platinum associated to DNA. No statistic difference in the associated platinum was found between a treated sample of DNA and an untreated one.

Platinum determination

Instrumentation. The atomic absorption spectrophotometer (Model 560, Perkin-Elmer) was equipped with a graphite furnace and ramp accessory (Model HGA 500, Perkin-Elmer) and an autosampler system (Model AS-1, Perkin-Elmer).

A platinum, hollow cathode lamp was the light source. The graphite furnace was supplied with pyrolytic-coated graphite tubes.

Operating conditions. The optimum parameters for platinum determination in the test solutions were: drying cycle, 100° for 40 sec with a temperature ramp of 40 sec, 140° with a temperature ramp of 20 sec, 400° for 10 sec with a temperature ramp of 10 sec; charring cycle, 1400° for 10 sec with a temperature ramp of 20 sec; and atomization cycle, 2700° for 6 sec with no ramping. The carrier gas was argon at a flow rate of 50 ml/min. Atomization peak heights were recorded at 265.9 nm, using a 3 × scale expansion. A 20 μl aliquot of the sample is injected into the furnace.

RESULTS

Resistant cell line

The level of the drug resistance was evaluated from 72 hr cell growth curves in the presence of serial dilutions of the drug and the drug sensitivities of cL and cLP cells were compared on the basis of their respective ID_{50} (i.e. the drug concentration reducing the number of live cells to 50% compared with drug-free cultures). The resultant ID_{50}s were 0.08 μg/ml for the cL and 2.5 μg/ml for the cLP which corresponds to a 30-fold increase of resistance acquired by the cLP cells (Fig. 1). As the parent cell line the resistant line displays a generation time of 12–14 hr in repeated experiments. Moreover the mean cell volumes (calculated as spheres with diameter values determined from camera lucida drawings on 50 cells) were respectively of 1594 μm^3 (SD = 495 μm^3) and

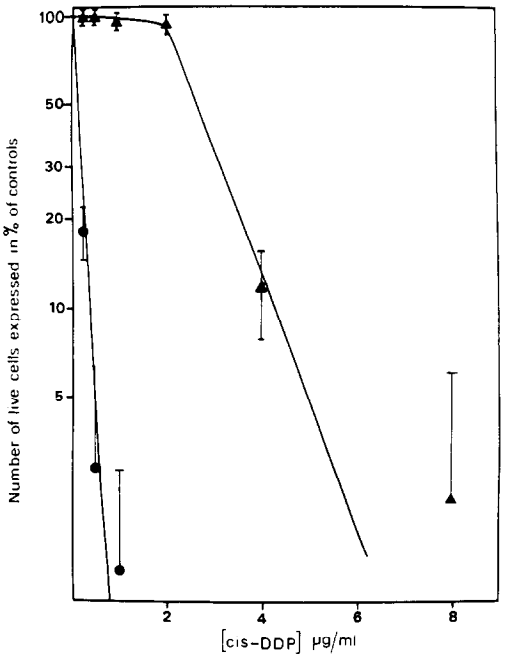

Fig. 1. Relative growth of the sensitive (—●—) and resistant (—▲—) cell lines. Cultures were seeded with 1.5×10^4 cells per ml and cells were counted after 72 hr of incubation with increasing concentrations of *cis*-DDP. Results are expressed in % of growth achieved in drug-free cultures controls. Error bars indicate the standard deviation.

1405 μm^3 (SD = 606 μm^3) for the cL and cLP cells. The corresponding nuclear volumes were 435 μm^3 (SD = 188 μm^3) and 324 μm^3 (SD = 169 μm^3) respectively. In both cases, the difference was found non-significant at 5%. The protein content of each line determined using the technique of Bradford [16] was 0.0156 mg/10^6 cells (SD = 1.45×10^{-3} mg/10^6 cells) and 0.0172 mg/10^6 cells (SD = 6×10^{-4} mg/10^6 cells) for cL and cLP respectively.

The mean survival time (±standard deviation) of recipient animals was compared: it reached 9.25 ± 0.25 days for the L1210 transplantable leukemia cells, 10.8 ± 0.6 days for the cL culture line and 13.8 ± 1.5 days for the cLP subline. Thus, the cultured cells retained a clear tumorigenic capacity. The delayed lethality in animals inoculated with the resistant cLP subline as compared with the lethality of animals bearing the sensitive cL line might result either from a possible slower *in vivo* growth of the cLP derivative (whereas the *in vitro* generation time is similar for the two lines) or from a relative decrease of tumorigenic potential related to the acquisition of the drug resistance. This latter interpretation is quite in line with numerous classical observations concerning a decreased or abolished tumorigenic potential in drug resistant sublines (actinomycin D, ellipticine, *cis*-DDP).

Determination of platinum associated to whole cells

Following incubation of sensitive cL and resistant cLP cells with *cis*-DDP in the presence of 0.9 µg/ml (3 µM) of drug, the amount of platinum associated to both sublines was followed as a function of time. Clear differences between the amounts of platinum associated respectively to the two cell lines were observed (Fig. 2). The amount of platinum associated to the resistant cells was lower than the amount associated to sensitive cells. This difference was specially pronounced after 6 hr of incubation. While the sensitive cells continued to accept the drug, the amount of platinum associated to the resistant cells reached a plateau at a level corresponding to about 0.15 ng of platinum per 10^5 cells.

We determined the amount of drug associated to whole cells after extensive washing which eliminated the platinum not strongly associated with its target. However, if in such conditions the platinum determination allows us to measure the amount of drug in the cell, it does not allow the discrimination between the drug attached to the outside of the membrane and (if any) the drug trapped inside the cells. Thus we cannot draw any conclusions con-

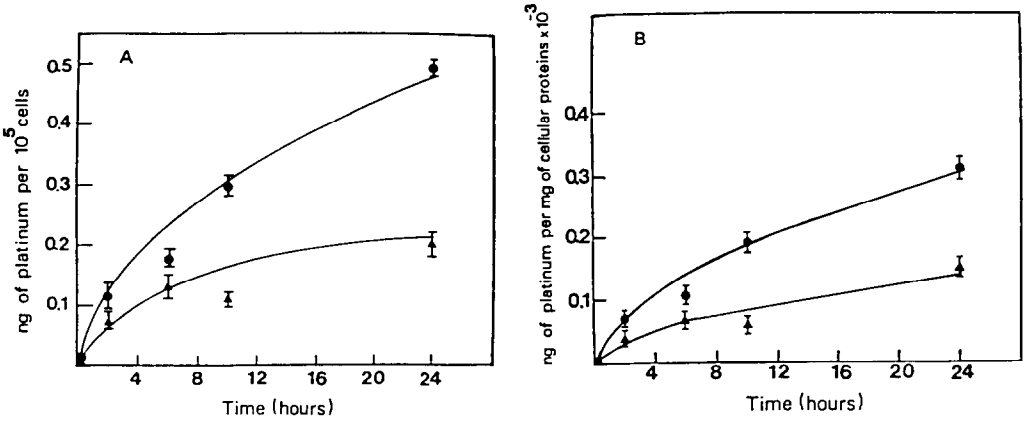

Fig. 2. Plots of *cis*-DDP (total concentration: 0.9 µg/ml) association to sensitive (—●—) and resistant (—▲—) L1210 cells. The associated platinum is expressed per 10^5 cells (panel A) and per mg of cellular proteins (panel B).

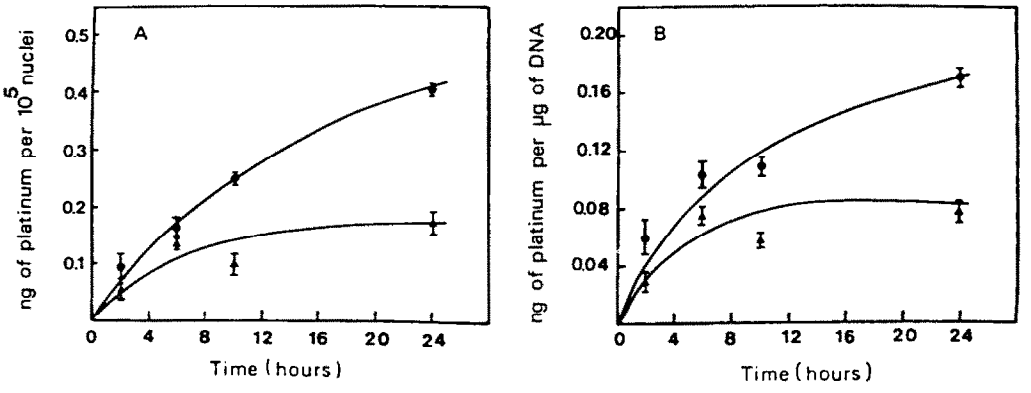

Fig. 3. Plots of the amount of platinum associated to nuclei after incubation of whole cells with *cis*-DDP. The associated platinum is expressed per 10^5 nuclei isolated from sensitive (—●—) or resistant (—▲—) cells in (A) and per μg of DNA (DNA content of the nuclei) in (B).

cerning the cell distribution of the drug. For this reason and in order to evaluate the amount of *cis*-DDP which indeed penetrated into the cells, we isolated the nuclei from platinum treated cells and measured the amount of platinum associated to this fraction.

Determination of platinum associated to nuclei

After cell treatment, performed in the same conditions as for the previous determination, we isolated the nuclei from these incubated cells and measured the amount of platinum associated to them. As shown in Fig. 3A, there is a difference between the two cell lines regarding the kinetics of nuclear association of *cis*-DDP. These results are quite comparable, both qualitatively and quantitatively, to our findings concerning the amount of drug present in whole cells. The nuclei isolated from drug-treated sensitive cells contained more drug than those isolated from resistant cells. Furthermore, in the resistant line, the amount of platinum after 8 hr reached a level identical to that obtained from whole cells. In the sensitive line, the association kinetics were about the same whether we measured the amount of platinum in the whole cells or in the nuclei. This indicates that the difference observed with the two kinds of cells is actually due to platinum content within the cells. In order to take into account a possible change in the DNA content of the nuclei isolated from sensitive cells compared to the nuclei isolated from resistant cells, we represent in Fig. 3B the amount of associated platinum normalized to the DNA content of the nuclei. One can notice that this representation gives the same type of kinetics, indicating that the resistant cells contain intrinsically less platinum than the sensitive ones. Moreover, by comparing the amounts of drug in the cells and in the nuclei, we can also conclude that the drug entering the cells reached the nuclei and was not trapped in the cellular membrane and/or in the cytoplasm. If the drug was trapped then we would have observed higher platinum content in whole cells than in nuclei alone.

Determination of platinum associated to DNA

Since DNA has been proposed as a target for *cis*-DDP action [4, 10], we isolated the DNA from sensitive and resistant cells after incubation with the drug and we measured the amount of platinum bound to it. The results are shown on Fig. 4. We observed a slight difference between the amounts of drug bound to the DNA extracted respectively from the sensitive and from the resistant cells for periods of incubation up to 6 hr. The *r* value (number of platinum atoms bound per DNA nucleotide) was about twice as high in the case of the sensitive as compared with the resistant cell line. When compared to the kinetics of *cis*-DDP binding to either the whole cells or to the isolated nuclei, the amount of DNA bound platinum did not reach a plateau in the case of the resistant cell line and continued to increase as a function of the incubation time. For incubation times longer than 6 hr the difference in the *r* values between the parental and resistant line tends to decrease reaching a value of about 1 platinum atom per about 17,000 base pairs for both cell lines. In order to check

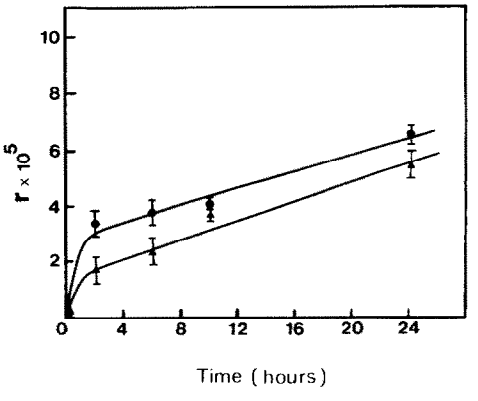

Fig. 4. Plots of the *r* values (bound platinum per DNA nucleotide) as a function of incubation time in sensitive (—●—) and resistant (—▲—) L1210 cells.

the possibility that the saturability of DNA did not bias our results, we further performed the evaluation of drug associated to DNA by incubating cells with higher amount of drug while keeping conditions of similar low drug toxicity levels (4.5 μg/ml, i.e. 15 μM for 1 hr). In these conditions, we found a ratio of about two between the amount of platinum associated respectively to the sensitive and to the resistant line thus similar to the ratio previously found: the values of r are than 6.6×10^{-5} for cL and 3.5×10^{-5} for cLP, values which should be compared to 2.4×10^{-5} (cL) and 1.3×10^{-5} (cLP) when the cells are incubated with 0.9 μg/ml (3 μM) of drug. This indicates that the low amount of drug bound to DNA is not a consequence of a limit in the saturability of the DNA in conditions where the cytotoxicity was of the same order for both cell lines.

DISCUSSION

In an attempt to understand further the mechanism of action of *cis*-DDP, we have compared the association of this drug to sensitive (cL) and resistant (cLP) cell culture lines derived from murine L1210 cells.

Our results show that the sensitive cell line contains more platinum than the resistant cell line and that the amount of platinum associated to the sensitive cell line is about three times higher than the amount associated to the resistant cell line.

However, shorter incubation times (up to 6 hr) yield less pronounced differences between the amounts of platinum in the sensitive or resistant cells.

In our conditions (3 μM *cis*-DDP) and for incubation times lower than 8 hr we do not observe any differences between the cell number plotted as a function of time for cL and cLP. This suggests that the observed differences in the *cis*-DDP content, in these conditions are not solely due to a difference of viability of the two cell lines. Nevertheless, we must consider that even if the number of cells for each line is not modified, a change in the membrane structure or in a transport system may have taken place leading to a slightly higher amount of platinum present in the sensitive line. Studies of L1210 cells resistant to *cis*-DDP have provided evidence that the drug is transported into the cells by an amino acid transport system [17–19].

These observations concerning the whole cell, led us to measure the amount of *cis*-DDP associated in the cell and specifically the level of drug in the nuclei and bound to DNA. We observed that the amount of platinum associated to the nuclei of both lines represents 83% and 75% of the platinum present in the cells, for cL and cLP respectively. We can therefore assume that the fraction of platinum trapped in the cell membrane and cytoplasm are respectively 17% and 25% of the total platinum in cL and cLP lines. This result agrees with the morphology of L1210 cells which contain a large nucleus surrounded by a relatively small cytoplasm. Along the same lines, a study concerning human carcinoma cells sensitive and resistant to *cis*-DDP, leads to the conclusion that in the sensitive line 64% of the bound platinum is located in the nuclei while 41% is located in the nuclei of the resistant line [20].

It is evident that once the drug gets into the cell, the major part of it is captured by nuclei. This has also been previously shown through electron microscopy [21]. These authors observe that *cis*-DDP is mainly found in the nucleolus and the inner side of the nuclear membrane.

Concerning the fraction of platinum associated to cytoplasm, it has been demonstrated that *cis*-DDP binds to metallothioneins, both *in vitro* and *in vivo* [22]. Furthermore, cells which have a high content of metallothioneins are resistant to *cis*-DDP [23].

We cannot exclude the hypothesis that an excess of metallothioneins or of other sulphydryl rich proteins could be present in the cytoplasm and/or in the nuclei of the cLP cell line.

Our results show that only 20% of the platinum present in the nuclei are bound to DNA. This binding is about the same for the two cell lines regardless of incubation time up to 24 hr.

The low amount of *cis*-DDP which is bound to DNA could correspond to DNA saturation in which chromatin structure prevents more platinum from binding to DNA. In this case, increasing the concentration of drug will not, in the same conditions of toxicity, change its quantitative binding. Indeed, when the cells are treated with 15 μM of drug for 1 hr (in which no apparent cytotoxicity is observed for both cell lines) we observe increases in the r values which are 6.6×10^{-5} and 3.5×10^{-5} for cL and cLP respectively. These values are about 3 times higher than the situation where the cells are incubated for 1 hr in the presence of only 3 μ of drug. The ratio between the r values corresponding to sensitive and resistant cells is 2 as in the previous case. It thus appears that the low amount of drug bound to the DNA as compared to the amount present in nuclei is the result of a preferential association of the drug to nuclear sites located outside the DNA. Furthermore, there is no direct correlation between the amount of platinum bound to the DNA of these cells and their sensitivity or resistance.

The strong difference we observe for the binding of platinum to the nuclei of either sensitive or resistant cell lines does not occur at the level of DNA. This suggests that even if the DNA is a critical target for *cis*-DDP, the binding is not the only important event leading to cell death.

Some nuclear proteins together with the RNA could be involved in the *cis*-DDP toxicity [24, 25]. The mechanism of resistance of cLP cells does not appear to be linked to the amount of platinum bound to DNA. This has already been suggested from previous studies which have shown that *cis* and *trans*-DDP react equally with DNA and that the difference between them is due to a specific type of lesion [4].

The mechanism of resistance to *cis*-DDP may therefore reflect a differential modification of DNA or a differential removal of DNA bound platinum in the two cell lines.

Other work concerning L1210 cells sensitive and resistant to *cis*-DDP shows that a correlation can be found between the amount of platinum induced interstrand DNA cross-links and the sensitivity to this compound [26]. On the other hand, Strandberg *et al.* [27] suggest that the mechanism of resistance could be the consequence of intrastrand cross-links.

In conclusion the mechanism of resistance of the cLP cell line does not imply a drastic quantitative difference in the amount of platinum bound to DNA as shown from direct measurements. The mechanism should be looked for in some other critical process, for example, the type of induced DNA cellular lesions.

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REFERENCES

1. Rozencweig M, Von Hoff DD, Slavik M and Muggia FM, *Cis*-diamminedichloroplatinum (II). A new anticancer drug. *Ann Inern Med* **86**: 803–812, 1977.
2. Gonia SL and Pizzo SV, Complexes of serium albumin and *cis*-dichlorodiammineplatinum (II). *J Biol Chem* **258**: 5764–5769, 1982.
3. Zwelling LA and Kohn KW, Mechanism of action of *cis*-dichlorodiammineplatinum (II). *Cancer Treat Rep* **63**: 1439–1444, 1979.
4. Roberts JJ and Thomson AJ, The mechanism of action of antitumor platinum compounds. *Prog Nucl Acid Res Mol Biol* **22**: 71–129, 1979.
5. Clear NJ, Some aspects of platinum complex chemistry and their relation to antitumor activity. *J Clin Hematol Oncol* **7**: 1–25, 1977.
6. Macquet JP and Butour JL, Modifications of the DNA secondary structure upon platinum binding: a proposed model. *Biochimie* **60**: 901–914, 1978.
7. Roos IAG, The interaction of an antitumor platinum complex with DNA. *Chem Biol Interact* **16**: 39–55, 1977.
8. Munchausen LL, The chemical and biological effects of cis-dichlorodiammineplatinium an antitumor agent on DNA. *Proc Natl Acad Sci USA* **71**: 4519–4522, 1974.
9. Shooter KB, Howse R, Merrifield RK and Robins AB, The interaction of platinum II compounds with bacteriophages T7 and R17. *Chem. Biol Interact* **5**: 289–307, 1972.
10. Beck DJ and Brubaker RR, Mutagenic properties of *cis*-platinum (II). *Mutat Res* **27**: 181–189, 1975.
11. Roberts JJ and Friedlos F, Quantitative aspects of the formation and loss of DNA interstrand crosslinks in Chinese hamster cells following treatment with *cis*-diamminedichloroplatinum (II) (cisplatin). I. Proportion of DNA-platinum reactions involved in DNA cross-linking. *Biochim Biophys Acta* **655**: 146–151, 1981.
12. Eastman A, Characterization of the adducts produced in DNA by *cis*-diamminedichloroplatinum (II) and *cis*-dichloro(ethylendiammine)-platinum (II). *Biochemistry* **22**: 3927–3933, 1983.
13. Micetich KC, Barnes D and Erickson LC, A comparative study of the cytotoxicity and DNA-damaging effects of *cis*-(diammino) (1,1-cyclobutanedicarboxylato)platinum (II) and *cis*-diamminedichloroplatinum (II) on L1210 cells. *Cancer Res* **45**: 4043–4047, 1985.
14. Zwelling LA, Anderson T and Kohn KW, DNA-protein and DNA interstrand cross-linking by *cis*- and *trans*-platinum (II) diamminedichloride in L1210 mouse leukemia cells and relation to cytotoxicity. *Cancer Res* **39**: 365–369, 1979.
15. Dhara SCA, Rapid method for the synthesis of *cis*-$Pt(NH_3)_2$-Cl_2. *Ind J Chem* **8**: 193–194, 1970.
16. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
17. Scanlon KJ, Safirstein RL, Thies H, Cross RB, Waxman S and Guttenplan JB, Inhibition of amino acid transport by *cis*-diammine-dichloroplatinum (II) derivatives in L1210 murine leukemia cells. *Cancer Res* **43**: 4211–4215, 1983.
18. Gross RB, Waxman S and Scanlon KJ, Amino acid membrane transport properties of L1210 cells resistant to cisplatin. *Chemotherapia* **5**: 37–42, 1986.
19. Shionoya S, Lu Y and Scanlon KJ, Properties of amino acid transport systems in K562 cells sensitive and resistant to *cis*-diamminedichloroplatinum (II). *Cancer Res* **46**: 3445–3448, 1986.
20. Teicher BA, Holden SA, Kelley MJ, Shea TC, Cucchi CA, Rosowsky A, Henner WD and Frei III E, Characterization of a human squamous carcinoma cell line resistant to *cis*- diamminedichloroplatinum (II). *Cancer Res* **47**: 388–393, 1987.
21. Khan MVA and Sadler PJ, Distribution of a platinum antitumor drug in HeLa cells by analytical electron microscopy. *Chem Biol Interact* **21**: 227–232, 1978.
22. Kraker A, Schmidt J, Krezoski S and Petering DH, Binding of *cis*-diamminedichloroplatinum (II) to metallothionein in Ehrlich cells. *Biochem Biophys Res Commun* **130**: 786–792, 1985.
23. Bakka A, Endresen L, Johnson ABS, Edminson PD and Rugstad HE, Resistance against *cis*-dichlorodiammineplatinum in cultured cells with a high content of metallothionein. *Toxicol Appl Pharmacol* **61**: 215–226, 1981.
24. Morris CR and Gale GR, Interactions of an antitumor platinum compound with deoxyribonucleic acid, histones, L-amino acids, poly-L-amino acids, nucleosides and nucleotides. *Chem Biol Interact* **7**: 305–315, 1973.
25. Waud WR and Blount SR, Biochemical studies on resistance to *cis*-platinum (*cis*-DDP) in L1210 leukemia. *Proc Am Assoc Cancer Res* **26**: 260, 1985.
26. Micetich K, Zwelling LA and Kohn KW, Quenching of DNA: platinum (II) monoadducts as a possible mechanism of resistance to *cis*-diamminedichloroplatinum (II) in L1210 cells. *Cancer Res* **43**: 3609–3613, 1983.
27. Strandberg MC, Bresnick E and Eastman A, The significance of DNA cross-linking to *cis*-diammine-dichloroplatinum (II) induced cytotoxicity in sensitive and resistant lines of murine leukemia L1210 cells. *Chem. Biol Interact* **39**: 169–180, 1982.