Differential Toxicity of cis- and trans-Diaminedichloroplatinum(II) toward Mammalian Cells: Lack of Influence of Any Difference in the Rates of Loss of Their DNA-bound Adducts

John J. Roberts and Frank Friedlos

Department of Molecular Pharmacology, Institute of Cancer Research, Clifton Avenue, Sutton, Surrey SM2 5PX, England

ABSTRACT

DNA-bound adducts formed by cis- or trans-diaminedichloroplatinum(II) (cis-DDP or trans-DDP, respectively) have very different effects on DNA synthesis and cell cycle progression in Chinese hamster cells. When the loss of platinum from the DNA of cells treated with pulsed doses of these isomers was corrected for the different effects they produced on DNA replication and cell cycle progression, then adducts formed by either agent were lost slowly from DNA and at comparable rates. The kinetics of accumulation of platinum on the DNA of exponentially growing Chinese hamster cells differed following continuous treatment with a low dose (10 μM) of either cis-DDP or trans-DDP. However, when these DNA binding values were again corrected for the different effects of the two isomers on DNA replication and cell cycle progression, both compounds reacted with DNA to similar extents. The amounts of platinum that accumulated by 30 h on the DNA of stationary-phase Chinese hamster cells treated with a low dose (10 μM) of either cis- or trans-DDP were also similar but resulted in very different effects on cell survival. The amounts of platinum accumulating on the DNA of near confluent African green monkey cells were also similar following continuous treatment with a low dose (10 μM) of either cis-DDP or trans-DDP, after correction for the relatively small amount of DNA synthesis occurring in these cells, and they were similar to the binding of platinum to the DNA of similarly treated Chinese hamster cells. There was no rapid loss of platinum from the DNA of African green monkey cells following treatment with pulsed low or high doses of trans-DDP. It could be concluded that the different cytotoxic effects produced by cis- or trans-DDP resulted from an intrinsic difference in the effects of their respective DNA-bound adducts on DNA replication and were not due to a difference in the rate of repair of such adducts, as previously proposed (R. B. Ciccarelli et al., Biochemistry, 24: 7533–7540, 1985). The accumulation of platinum on the proteins of Chinese hamster or African green monkey kidney cells treated with cis- or trans-DDP was also consistent with the respective toxic effects of the two isomers.

INTRODUCTION

cis-DDP (cisplatin) is a useful antitumor drug whose cytotoxic action is thought to be due to its ability to react with DNA (see Refs. 1 and 2). The corresponding trans isomer, trans-DDP, on the other hand, is not an antitumor agent and is less toxic to mammalian cells in culture (3). The higher doses of trans-DDP required to produce effects equitoxic to those produced by cis-DDP produced correspondingly higher levels of reaction with DNA (approximately 5 to 10 times) in both HeLa cells (3) and Chinese hamster cells (4). However, treatment of HeLa cells with equitoxic doses of the two compounds produced approximately equal amounts of cross-linking of opposite strands of cellular DNA (3, 5). The greater capacity of the cis compound to produce these DNA interstrand cross-links, as compared with the trans compound, has been observed in Chinese hamster (6, 7) and mouse cells (8, 9). Moreover in studies of both mouse leukemia cells (10) and normal and transformed human cells (11, 12) of differing sensitivity to cis-DDP it was shown that this sensitivity correlates well with interstrand cross-link formation. These various observations therefore strongly indicated that the ability to form DNA interstrand cross-links was causally related to the toxic effects of the neutral platinum coordination compounds, and also provided a satisfactory explanation for the greater cytotoxic activity of the cis isomer.

On the other hand, other studies during the past few years have added evidence that the major adduct formed in DNA by cis-DDP, but not by the trans isomer, is an intranuclear cross-link between two adjacent guanines on one strand of DNA (see Ref. 13) and that this difference adequately accounts for the antitumor activity of the former but not of the latter. In addition a recent report claimed that DNA adducts produced by the two compounds are repaired differently in African green monkey CV-1 cells, as indicated by their different kinetics of accumulation of platinum on, or disappearance from, DNA (14). However, these latter studies did not take into account a possible difference in the effects of the two agents on the rate of replication of DNA and subsequent growth of cell populations which might adequately explain the observed difference in the rate of removal of adducts from the DNA of cells following their treatment with the cis- or trans isomer.

Previous studies have established that adducts formed by cis-DDP are slowly lost from the DNA of Chinese hamster cells (15), presumably by a DNA excision repair process (16); but, hitherto, the rate of loss of trans-DDP-induced DNA adducts has not been reported in these cells. Likewise the effects of the cis-DDP adducts on DNA replication in both prokaryotes and eukaryotes have been described (see Refs. 1 and 2), but the effects of adducts formed by the trans isomer have not been completely defined in mammalian cells. Conceivably also, African green monkey cells differ from Chinese hamster cells with respect to their response to DNA-bound platinum. We now report that, at equivalent levels of reaction with DNA, DNA synthesis is indeed markedly more inhibited by cis-DDP as compared with trans-DDP in both African green monkey and Chinese hamster cells and that, when account is taken of such a difference, adducts formed by the two isomers are removed from the DNA of both cell types at comparable rates.

MATERIALS AND METHODS

Growth and Labeling of Cells. The DNA of Chinese hamster V79 cells, growing in stirred suspension culture (17), was labeled by growth for 24 h in the presence of [2-14C]thymidine (Amersham International) (50 μCi/mmol; 10 nCi/ml) followed by a further 16-h growth in label-free medium, to a specific activity of between 0.1 and 1.0 μCi/mg of DNA. Cultures intended to be in stationary phase at the time of exposure to the platinum compound were grown to a cell density of 10⁶/ml during the labeling and label-free period of growth and were maintained at this density for a further 24 h prior to use. Cultures

Received 6/19/86; revised 9/18/86; accepted 9/30/86.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Financial support from the Johnson Matthey Research Centre and grants from the Medical Research Council and Cancer Research Campaign.

2 The abbreviations used are: DDP, diaminedichloroplatinum(II); PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide.
intended to be in exponential phase were diluted to $4 \times 10^3$/ml and used immediately.

African green monkey kidney CV-1 cells were grown as a monolayer in Eagle's minimal essential medium supplemented with 1% nonessential amino acids and 10% fetal calf serum under which conditions they doubled every 48 h. Their DNA was labeled by 7 to 10 days of growth in the presence of $[2^{-14}C]$ thymidine (50 mCi/mmol; 12 nCi/ml), followed by 2 days of growth in label-free medium to a $^{14}C$ specific activity of 1 to 10 nCi/mg.

Treatment with Platinum Compounds. Bulk cultures of V79, or replicate bottles of CV-1, cells were exposed to cis-DDP or trans-DDP by addition of a freshly prepared 100-fold concentrate in dimethyl sulfoxide and, for some experiments, in PBS to give either a 10 μm continuous treatment or a 2- or 4-h pulsed treatment with the doses stated. Control (untreated) cultures received a solvent blank only. Either immediately after addition of the platinum compounds or after termination of the pulsed exposure, and at times thereafter, bottles of CV-1 cells or aliquots of the V79 cultures sufficient to yield at least $2 \times 10^6$ cells were harvested, washed in ice-cold PBS, and flash frozen at $-80^\circ$C. Throughout the time course of the experiment V79 cell numbers were monitored, and where indicated, cell samples were removed for estimation of platinum-induced cytotoxicity by a colony-forming assay, as previously described (17).

Isolation of DNA and Protein and Estimation of Specific Radioactivity and Amount of Bound Platinum. The frozen cell pellets were thawed into a p-aminosalicylic acid lysing solution (18) and extracted with a phenol mixture, and the DNA was precipitated by the addition of 2-ethoxyethanol and washed with ethanol. The DNAs were redissolved in distilled water to about 0.5 mg/ml and further purified by digestion with RNase (bovine pancreatic RNase A, type 1-AS; Sigma) followed by proteinase k (Sigma), both at 50 μg/ml for 30 min at 37°C, and then reextraction with the phenol mixture. DNA was reprecipitated with 2-ethoxyethanol and washed successively with ethanol (75%), ethanol, and ether and finally dried in a vacuum desiccator for 16 h. The protein fractions were harvested by diluting a sample of the initial phenolic phase with a large excess of a methanoheter mixture (3:1). The precipitated protein was washed 3 times with ether and then vacuum desiccated as for the DNA. All purified, dried DNA weights were in excess of 1 mg.

The dried DNA or protein samples were individually weighed and hydrolyzed by digestion in concentrated hydrochloric acid at 60°C for 1 h at a concentration of 30 mg/ml. Aliquots of the DNA hydrolysate were removed, neutralized, and assayed for $^{14}C$ radioactivity by liquid scintillation counting and DNA content by UV spectrophotometry, thus providing a measure of specific $^{14}C$ activity of the DNA during the course of the experiments. Both the remaining DNA and protein hydrolysates were assayed for DNA content by flameless atomic absorption spectrophotometry in a Perkin-Elmer Model 306 spectrometer, equipped with a continuum-method background corrector and stopped gas flow, and an HGA72 heated graphite atomizer. Hydrolysate volumes corresponding to greater than 1 mg of DNA were used which generally gave absorbance peaks of 0.1 absorbance unit or greater, indicating amounts of platinum per determination in excess of 10 ng.

RESULTS

Studies of Chinese Hamster Cells

Effect of a Pulse Treatment with trans-DDP on DNA Synthesis in Chinese Hamster V79 Cells. Exponentially growing Chinese hamster cells, prelabeled in their DNA and treated with 100 μm trans-DDP for 2 h, continued to synthesize DNA during the following 30 h at a rate comparable to that in control, untreated cultures. Thus the specific activity of the DNA from control cultures decreased with time after treatment with a half-life of about 13 h (Fig. 1B) (consonant with the known doubling time of these cells), while the specific activity of DNA from similarly labeled cells treated with trans-DDP decreased with a half-life which was only increased to 14.7 h (Fig. 1).

Binding of Platinum to the DNA or Protein of Chinese Hamster V79 Cells with Time after Pulse Treatment with trans-DDP. The specific binding of platinum to the DNA of Chinese hamster cells immediately following their treatment with 100 μm trans-DDP for 2 h was 244 nmol/g. As seen in Fig. 1, the specific binding of platinum to DNA decreased during 30 h at a rate ($t_0 = 11.7$ h) only slightly greater than that found for the decrease in the $^{14}C$ specific activity of the same DNA, indicating that the majority of the decrease of specific platinum binding is due to the increasing amount of DNA present rather than to loss of platinum from the original DNA. When the specific platinum binding at later times is adjusted to take account of this increase in amount of DNA actually present, then it can be calculated that trans-DDP bound platinum is lost only slowly from DNA and that 60 to 70% of the platinum is still present on the DNA 30 h after treatment.

The specific binding of platinum to protein was initially 517 nmol/g and decreased during 30 h after treatment with a half-life of 10.4 h, indicative not only of cell cycle progression (unimpeded by the trans-DDP treatment), but also metabolic turnover of protein during the cell cycle.

The slow rate of loss of platinum from the DNA of trans-DDP-treated cells could possibly have been due to inhibition or saturation of any hypothetical DNA excision repair process(es) following high levels of reaction with DNA. We therefore examined the time course of the binding of platinum to DNA after continuous treatment of cells with a low dose of cis- or trans-DDP in case it revealed evidence for the rapid loss of trans-DDP-induced adducts as apparently observed previously by others (14), although we appreciate that such conditions are not ideal for studying the repair of platinum-induced DNA damage. The insensitivity of atomic absorption for detecting DNA bound platinum did not permit us to use a low pulsed dose of trans-DDP to study DNA repair.

Effect of a Continuous Exposure to cis-DDP or trans-DDP on the DNA Synthesis and Cell Population Growth of Chinese Hamster Cells. Exposure of exponentially growing Chinese hamster cells to a continuous treatment with 10 μm trans-DDP also produced only minimal effects on cell cycle progression (Fig. 2A) and DNA synthesis (Fig. 2B). Thus the specific activity of $^{14}C$-labeled DNA from the control, untreated cells decreased apparently exponentially, halving in value after about 12 h, while that from cultures treated with trans-DDP halved in about 14 h. By contrast, DNA synthesis in the cis-DDP-treated cultures is markedly inhibited (Fig. 2B). The specific activity of the $^{14}C$-labeled DNA decreased, again apparently
Differential Toxicity of cis- and trans-DDP Toward Mammalian Cells

are therefore not able to comment on the precise kinetics of binding of platinum to DNA during the experiment. Clearly the kinetic values are complex and probably represent changing proportions of intermediate platinum derivatives of varying reactivities towards DNA. However, irrespective of perturbations in binding resulting from these factors the overall binding of platinum to DNA increases for both compounds throughout the experimental period, and there is no evidence for the rapid loss of trans-DDP adducts from cellular DNA at late times after treatment, as observed in other studies (14).

The specific binding of platinum to the total protein of cells at various times after treatment with cis- or trans-DDP is shown in Fig. 3. The binding to protein during exposure to trans-DDP is seen to peak at about 2 h and then decline rapidly, whereas, during exposure to cis-DDP, the peak of specific binding is reached later at 10 h, and the rate of decline subsequent to this is initially slower. These findings are consistent with an initially faster rate of reaction of the trans isomer and with the above differences in DNA replication and cell cycle progression in cell cultures treated with the two isomers (Fig. 2, A and B).

Time Course of Accumulation of Platinum on the DNA or Protein of Stationary-Phase Chinese Hamster Cells Exposed to Continuous Treatment with cis- or trans-DDP. In order to confirm that the above decrease in amount of platinum bound to the DNA of trans-DDP-treated cells at late times after treatment was indeed due to the continued synthesis of DNA and continued cell division and not to selective repair of trans-DDP adducts, we treated an essentially non-DNA synthesizing, stationary-phase culture with cis- or trans-DDP and again measured the amount of platinum present on the DNA with time after treatment. The specific activity of 14C-labeled DNA remained essentially static during the experimental period, confirming the stationary nature of the cultures.

The pattern of accumulation of platinum on the DNA of stationary-phase cells treated with cis- or trans-DDP is illustrated in Fig. 4 (after adjustment for the small amount of DNA synthesis which was shown to have taken place). The rate of reaction of trans-DDP with DNA is initially faster than that of cis-DDP, but by 30 h after treatment both compounds had reacted to essentially the same extent, namely 140 nmol/g, a value similar to that found for the binding of both compounds to the DNA of exponentially growing cells. Again there was no evidence for any rapid loss of trans-DDP adducts from DNA (such as would be indicative of their selective repair) as compared with cis-DDP adducts.

The profiles for the time courses of binding of platinum to the proteins of stationary-phase cells treated with either cis-DDP or trans-DDP (Fig. 5) are somewhat different from those exponentially, but at a much slower rate, halving in value only after about 33 h. This difference in the effects of cis- and trans-DDP on DNA synthesis is further reflected in the cell culture growth profiles during the course of the experiment. Thus as seen in Fig. 2A, the rate of increase in cell number of the control culture is barely distinguishable from that of the trans-DDP-treated culture, both doubling in cell number in about 12 h. The cis-DDP-treated culture, on the other hand, did not double in cell number within the duration of the experiment.

Time Course of Accumulation of Platinum on the DNA or Protein of Exponentially Growing Chinese Hamster Cells Exposed to a Continuous Treatment with cis- or trans-DDP. During the exposure of Chinese hamster cells to 10 μM cis-DDP platinum adducts accumulate on their DNA in a progressive manner plateauing, by 40 h after treatment, at a binding level of around 75 nmol/g (Fig. 2C). By contrast, during exposure of cells to trans-DDP, plateauing at about 30 nmol/g. When, however, these values for the specific platinum binding to DNA are adjusted for the different amount of DNA synthesis which has taken place during the course of the reaction period with either the cis- or trans-DDP (Fig. 2B) (i.e., multiplied by the DNA dilution factor), then a different pattern of binding to DNA emerges. Thus, as shown in Fig. 2D, the time course of reaction of cis-DDP or trans-DDP with cellular DNA both show a continuous increase in the binding of both compounds to DNA to reach levels of about 120 to 140 nmol/g by 35 h after treatment. There is some scatter in the values shown in Fig. 2D, since they represent the ratio of two values, the specific activity of 14C-labeled DNA and the specific platinum binding, both of which have inherent errors in their determination. We

![Graph showing the time course of platinum binding to DNA](image-url)
cells observed only a small decrease (about 25%) in the specific activity of the 
14C-labeled DNA of control cells during the 50 h of the experiment, consistent with the doubling time of 2 days found for these cells and the near-confluent nature of our cell cultures. Also, as found previously, trans-DDP had little effect on this DNA synthesis, but it was markedly inhibited by cis-DDP (data not shown). In view of this minimal amount of DNA synthesis occurring in these cells the patterns of platinum binding to DNA after treatment with cis- or trans-DDP were similar either before or after correction for effects on DNA replication, and we therefore show only data for the binding to DNA after correction for such DNA synthesis as does occur. Both agents react continuously with DNA for up to 50 h after their addition (Fig. 6). Moreover the levels of reaction with DNA reached after 30 h were similar for both compounds and of the same order as observed in similarly treated Chinese hamster cells (Figs. 2D or 4). The kinetics of binding of trans-DDP to DNA observed in this experiment is therefore markedly different from that previously described by Ciccarelli et al. (14). Again, as for similarly treated Chinese hamster cells, there was no evidence for any gross differences in the loss of DNA adducts produced by cis- or trans-DDP, suggestive of preferential repair of trans adducts, and such as could account for their very different biological effects.

Binding of Platinum to DNA of CV-1 Cells following a Pulse Treatment with trans-DDP. Ciccarelli et al. (14) reported a dramatic decrease in the binding of platinum to the DNA of CV-1 cells, from a peak value at around 6 h after treatment, during the following 6 h, in cells being treated continuously with 10 μM trans-DDP. We have therefore measured the binding of platinum to the DNA of CV-1 cells after a 4-h treatment with 10 μM trans-DDP and then subsequently after a further 6- h incubation in the absence of trans-DDP. As seen in Fig. 6, the amount of platinum bound to DNA after 4 h of exposure to trans-DDP did not change appreciably during the following 10 h. In the experiments reported by Ciccarelli et al. (14) trans-DDP was dissolved in saline rather than in DMSO, as in the experiments reported herein. It is known that DMSO can react with trans-DDP to give DMSO-substituted compounds and that these compounds can react differently from trans-DDP with DNA in vitro. However, our DMSO solutions were diluted 100-fold in medium within 5 min of preparations and are therefore unlikely to contain any appreciable quantity of DMSO-substituted adducts. (DMSO reacts with trans-DDP with a half-life of 19 min at 37°C.) Moreover, we obtained the same effect on the cell killing of Chinese hamster cells irrespec-
Differential Toxicity of cis- and trans-DDP Toward Mammalian Cells

Fig. 7. Specific platinum binding to the proteins of African green monkey kidney cells with time after continuous treatment with 10 μM cis-DDP (■) or trans-DDP (▲).

The binding of platinum to the proteins of trans-DDP-treated CV-1 cells reached a peak about 6 h after treatment (Fig. 7), before steadily declining. The pattern of protein binding to CV-1 cells is therefore also comparable to that seen in trans-DDP-treated Chinese hamster cells. These effects are consistent again with the lack of effect of trans-DDP on protein synthesis or cell cycle progression.

Discussion

In this paper we have attempted a critical examination of the recent postulate that differential repair of the DNA lesions produced by cis- or trans-DDP could account for the greatly increased toxicity of the cis versus the trans isomer (14).

(a) Therefore, we have investigated the effect of cis-DDP or trans-DDP on DNA synthesis and cell cycle progression in both Chinese hamster and African green monkey kidney cells as a function of the dose of the agent and of the amount of platinum actually bound to the DNA, since a knowledge of these effects is critical to an interpretation of data on the binding and possible loss of platinum-induced lesions on DNA. We have shown that trans-DDP has little effect on DNA synthesis whether administered as a 2 h pulse of 100 μM to Chinese hamster cells or as a continuous exposure of 10 μM to Chinese hamster or African green monkey kidney cells. By contrast, continuous treatment with 10 μM cis-DDP dramatically inhibited DNA replication in both cell lines. We have also shown that there was no significant difference in the final extent of binding of platinum to DNA with the two agents, irrespective of whether cells were growing exponentially or were in stationary phase (Figs. 2 and 4). Therefore the observed differential effect on DNA synthesis at early times after treatment by cis or trans is not a function of different cellular uptake or reaction with DNA but due to an intrinsic difference in the consequences of the different types of DNA adducts formed by the two isomers on DNA replication, while the markedly greater effect of cis-DDP at later times after treatment is due to a combination of this effect and to the subsequent marked block to cell cycle progression induced by the cis but not by the trans isomer.

Previous studies have demonstrated that cis-DDP can selectively inhibit DNA replication in human AV3 (19), HeLa (2), and Chinese hamster (4, 7, 20) cells in culture and Ehrlich ascites cells in vivo (21). By comparison, a trans-platinum(IV) compound was found to be much less effective at inhibiting DNA replication in AV3 cells (19). While both cis- and trans-DDP can inactivate primed templates as substrates for DNA polymerase α and β (22) and polymerase I (23) and with apparently equal efficiency, cis-DDP-induced adducts are 5 times more effective than trans-DDP-induced lesions at inhibiting DNA replication on templates of T7 DNA (4). This latter system therefore reflects the relative effectiveness of the two platinum compounds on the replication of mammalian cell DNA, as observed in this study.

(b) We have examined the loss of trans-DDP-induced adducts from mammalian cell DNA and in isolation from the perturbing influences of both further DNA reaction and DNA replication (Fig. 1) and shown that this proceeds only slowly, being comparable to the previously found rate of loss from DNA of cis-DDP-induced adducts (15). Thus we were not able to find evidence for exceptionally rapid repair of trans-DDP-induced, but not of cis-DDP-induced adducts, as proposed by Ciccarelli et al. (14). It is also apparent that the slow excision of trans-DDP-induced DNA adducts takes place in cells that are actively replicating their DNA and capable of further cell division, whereas the loss of cis-DDP-induced adducts occurs from nonreplicating DNA and during a pronounced cytostatic period. Thus DNA replication takes place on a template containing trans-DDP adducts which do not have to be excised in order for replication to proceed.

(c) We studied the accumulation of platinum on the DNA or protein of exponentially growing or stationary-phase Chinese hamster cells exposed to a continuous low dose of cis- or trans-DDP [the treatment protocol used by others (14)] in case these treatment conditions resulted in different cellular responses from those seen above in cells treated with single large doses of the two platinum compounds. Conceivably continuous exposure to low doses of trans-DDP could induce mechanisms for repairing DNA-bound adducts. trans-DDP appears to react with both DNA and protein faster than does the cis isomer. Since the reaction approaches completion within the time course studied the reaction of the trans compound with cellular DNA peaks and plateaus sooner than that of the cis compound. By contrast the binding of the cis compound results in a simpler, single component curve. These DNA-binding profiles therefore
show a considerable resemblance to those described by Ciccarelli et al. (14) for reaction with the DNA of African green monkey cells. However, whereas these latter authors attributed the difference between the DNA-binding profiles for cis- or trans-DDP to the repairability of the respective DNA lesions, our data show that these are lost about equally, and slowly when compared to the above processes, from cellular DNA. Moreover, when the above multiphase profiles are resolved for the diluting effect of DNA synthesis the DNA-binding profiles for both cis- or trans-DDP become virtually coincident. Thus the finding of Ciccarelli et al. (14) could possibly be explained in terms of the different reactivities of cis- and trans-DDP toward cellular DNA [as characterized by Johnson et al. (4)] and the subsequent differences in the effects of their respective DNA adducts upon DNA synthesis and cell growth. The very different effects on subsequent cell survival of equivalent extents of binding by the two isomers to the DNA of stationary-phase V79 cells therefore confirmed earlier similar findings in Hela (3) and Chinese hamster ovary (4) cells, and they further emphasize the different toxic consequences of the DNA adducts formed by cis- or trans-DDP.

(d) We investigated the possibility that Chinese hamster cells, as used for our initial studies of the binding of platinum to DNA, may differ from African green monkey kidney cells with respect to their DNA repair characteristics. It is apparent, however, that very similar results were obtained for both cell types, further indicating that trans-DDP-induced DNA lesions are not lost more rapidly from cellular DNA than are cis-DDP-induced lesions. The kinetics of binding of cis- or trans-DDP to DNA we observed in Chinese hamster cells, prior to correction for effects on DNA synthesis (Fig. 2) resembled that described by Ciccarelli et al. (14) in African green monkey cells. On the other hand, in our experiments using these cells we failed to observe the same kinetics of DNA binding as reported by these authors or to obtain any evidence for repair of trans-DDP-induced adducts. We are unable to offer a satisfactory explanation for their apparent indication of repair of trans-DDP-induced DNA damage in CV-1 cells.

In conclusion, our data further indicate the probable importance of reactions with DNA by the antitumor platinum compounds in inducing cytotoxic effects in mammalian cells. The two isomeric adducts are removed from the DNA at the same rate, and it is the ability of such DNA adducts to block DNA replication, in preference to blocking other biochemical pathways, that is a prerequisite for cytotoxic action and accounts for the greater toxicity of the cis as compared to the trans isomer.

Current knowledge of the chemistry of the interaction of cis- or trans-DDP with cellular DNA does not permit any definite conclusions with regard to which of the many lesions they induce in DNA accounts for their different biochemical effects. Thus, while cis-DDP is more effective than trans-DDP at inducing interstrand cross-links in DNA (see Refs. 1 and 2), it can also more readily induce links between adjacent guanines on the same strand of DNA than can trans-DDP (see Refs. 2 and 13). Hence either type of cross-link is a possible candidate for the lesion that most effectively blocks DNA replication, although the net effect of a given lesion upon any biochemical process will be the product of its intrinsic (molar) effectiveness and its frequency with respect to total lesions.

ACKNOWLEDGMENTS

We would like to thank Sylvia Stockbridge and Rosemary Couch for preparing the manuscript.

REFERENCES

Differential Toxicity of cis- and trans-Diamminedichloroplatinum(II) toward Mammalian Cells: Lack of Influence of Any Difference in the Rates of Loss of Their DNA-bound Adducts

John J. Roberts and Frank Friedlos


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/1/31