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INTERACTIONS BETWEEN MAMMALIAN CELL DNA AND INORGANIC PLATINUM COMPOUNDS—II

INTERSTRAND CROSS-LINKING OF ISOLATED AND CELLULAR DNA BY PLATINUM (IV) COMPOUNDS

JANET M. PASCOE and JOHN J. ROBERTS

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London SW3 6JB, England

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Abstract—Measurement of the effects of *cis* and *trans* Pt(IV)diammine tetrachloride on the colony-forming ability of HeLa cells showed that the *cis* isomer was much more toxic than the *trans* on a dose basis. Attempts were made to correlate the ability of *cis* and *trans* Pt(IV)-diammine tetrachloride to cross-link isolated and cellular DNA with their observed cytotoxic properties. However the differences seen *in vitro* and *in vivo* were approximately the same, and neither was large enough to account for the observed difference in toxicity of the two compounds towards HeLa cells. It is therefore concluded that it is unlikely that the ability of *cis* and *trans* Pt(IV)diammine tetrachloride to cross-link isolated or cellular DNA bears any relationship to their cytotoxic properties.

Cis AND *trans* Pt(IV)diammine tetrachloride have been shown to differ in their ability to force filament formation in bacteria,[1] inhibit DNA, RNA and protein synthesis in mammalian cells[2] and arrest tumour growth in whole animals.[3] As in the case of the platinum(II) compounds, the *cis* isomer was more active than the *trans* in causing these effects. The activity of *cis* Pt(IV)diammine tetrachloride against various experimental animal tumours carried in mice,[3–5] and rats[6] indicates that it could be a useful chemotherapeutic agent. Available evidence indicates that the cytotoxic activities of the platinum(IV) compounds are probably the direct result of their reaction with DNA[2] rather than the consequence of RNA- or protein-mediated reactions.

Using cultured HeLa cells, the present study has thus sought to establish whether or not the difference in ability of *cis* and *trans* Pt(IV)diammine tetrachloride to form interstrand cross-links in isolated or cellular DNA could be correlated with the observed variation in their cytotoxic properties.

MATERIALS AND METHODS

Cis Pt(IV)diammine tetrachloride was provided by Professor M. L. Tobe and the *trans* isomer by Professor B. Rosenberg.

HeLa cells were cultured, and the effects of platinum(IV) compounds on their survival determined according to the protocol given in the first paper of this series.[7]

DNA was extracted from HeLa cells, and the extent of interstrand cross-linking was measured as previously described.[7]

RESULTS

Effects on cell survival. HeLa cell survival was reduced by the amounts shown in Fig. 1 following a 2 hr treatment with either *cis* or *trans* Pt(IV)diammine tetrachloride. D_Q (the intercept dose) and D_0 (the dose increment required to bring about an average of 1 lethal event per cell once the exponential portion of the survival curve

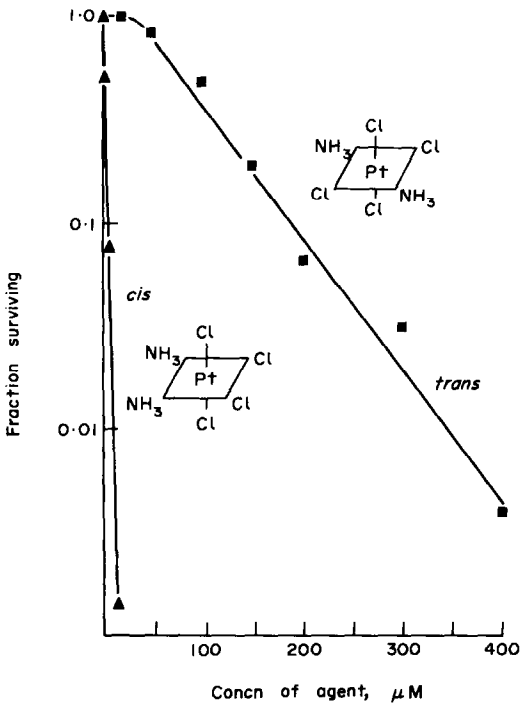

FIG. 1. Survival curves for treatment of HeLa cells in suspension culture with either *cis* Pt(IV)diammine tetrachloride (▲), or *trans* Pt(IV)diammine tetrachloride (■), for 2 hr at 37°.

had been reached)[8] values obtained from these survival curves are given in Table 1. It is apparent from these values that much more *trans* Pt(IV)diammine tetrachloride than the *cis* isomer must be given to HeLa cells in order to produce the same effect on cell survival.

As in the case of the platinum(II) compounds,[7] the *cis* isomer is more toxic to HeLa cells than the *trans* compound, and these observations are in agreement with those made in bacterial and whole animal systems.[1-6]

DNA cross-linking. Figure 2 shows that the difference in the ability of *cis* and *trans* Pt(IV)diammine tetrachloride to cross-link DNA *in vitro* is not great. The *cis* compound causes about 1·5 times as much cross-linking as the *trans* isomer at the lowest dose investigated, but only 1·1 times as much at the highest dose. This variance is of the same order as that observed for *cis* and *trans* Pt(II)diammine dichloride under the same experimental conditions.[7] However the platinum(IV) compounds are considerably less reactive than their platinum(II) counterparts in this system, for as can be seen from Fig. 3, 50 times as much of the platinum(IV) compounds are required

TABLE 1.

Compound	D_Q (μM)	D_0 (μM)
Cis $Pt(IV)(NH_3)_2Cl_4$	1·0	1·5
Trans $Pt(IV)(NH_3)_2Cl_4$	37·5	65·0

to produce the same level of cross-linking as observed with *cis* and *trans* Pt(II)diammine dichloride. As was the case with the platinum(II) compounds,[7] the difference in ability of *cis* and *trans* Pt(IV)diammine tetrachloride to cross-link isolated DNA is not great enough to explain the disparity in their cytotoxic properties. In view of the possibility that the environment of DNA within the whole cell could alter its reactivity towards platinum compounds, the ability of *cis* and *trans* Pt(IV)diammine tetrachloride to cross-link cellular DNA was measured.

As can be seen from Fig. 4 the 1 2-fold difference in the ability of *cis* and *trans* Pt(IV)diammine tetrachloride to form interstrand cross-links in the DNA of whole HeLa cells is much the same as that observed on treatment of isolated DNA (Fig. 2). These results are to be contrasted with those obtained for the platinum(II) compounds, which differed far more in their ability to cross-link cellular DNA than they did in their ability to cross-link isolated DNA. This is apparent from Fig. 5. Furthermore, Fig. 5 shows that the abilities of platinum(II) compounds and platinum(IV) compounds to form interstrand cross-links in cellular DNA are of the same order. This is in contrast to the situation obtaining *in vitro* where the platinum(IV) compounds were very much less reactive than their platinum(II) counterparts.(Fig. 3).

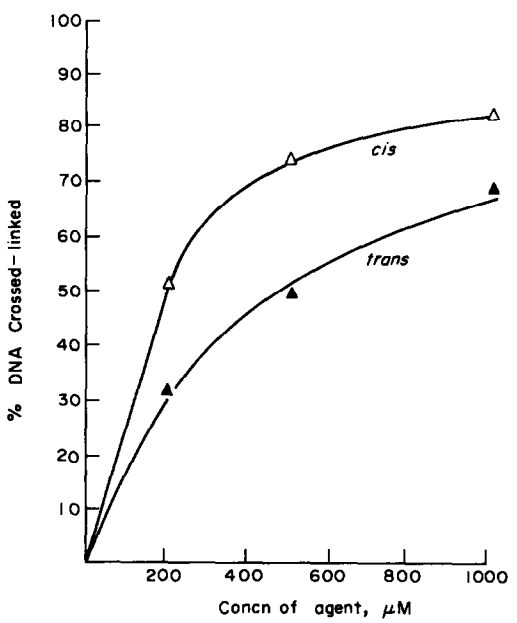

FIG. 2. Proportion of isolated HeLa cell DNA cross-linked by *cis* Pt(IV)diammine tetrachloride (△) and *trans* Pt(IV)diammine tetrachloride (▲) following treatment in neutral phosphate buffer for 2 hr at 37°.

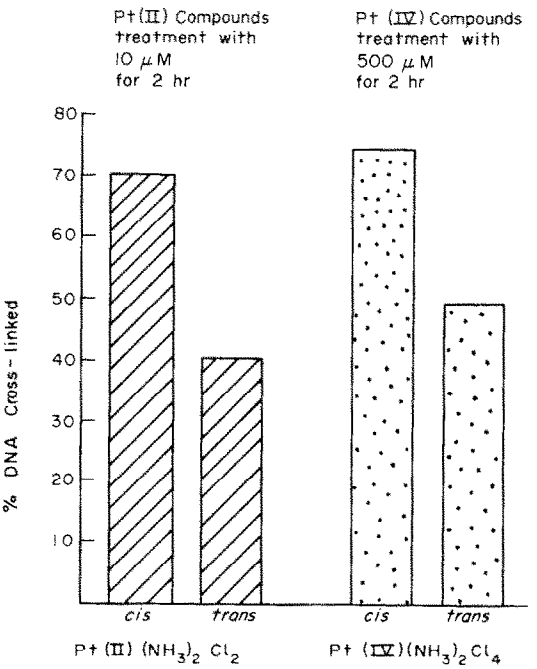

FIG. 3. Comparison of the abilities of platinum II compounds and platinum IV compounds to cross-link isolated DNA. Proportion of isolated HeLa cell DNA cross-linked by 10 μM *cis* Pt(II)diammine dichloride, 10 μM *trans* Pt(II)diammine dichloride, 500 μM *cis* Pt(IV)diammine tetrachloride and 500 μM *trans* Pt(IV)diammine tetrachloride, following treatment in neutral phosphate buffer for 2 hr at 37°.

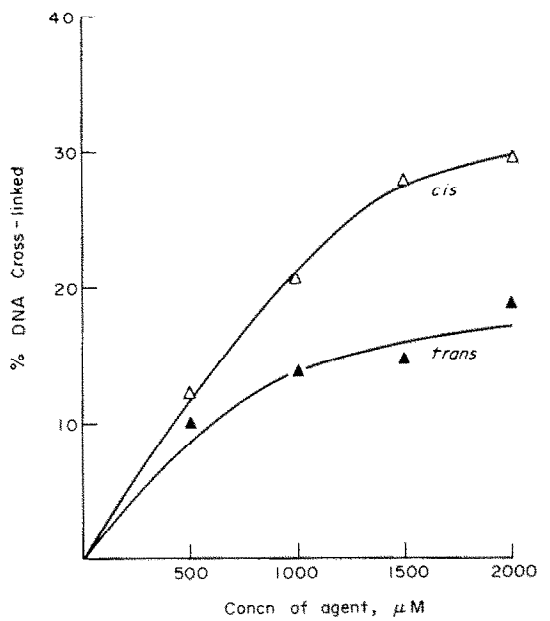

FIG. 4. Proportion of DNA cross-linked by *cis* Pt(IV)diammine tetrachloride (△), and *trans* Pt(IV)diammine tetrachloride (▲) following treatment of HeLa cells in suspension culture for 2 hr at 37°.

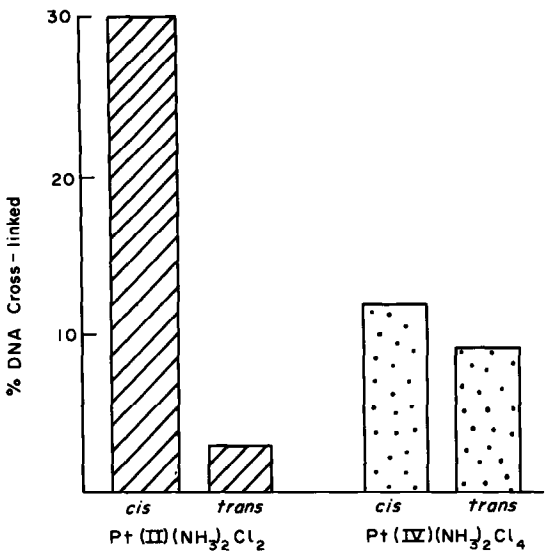

FIG. 5. Comparison of the abilities of platinum II compounds and platinum IV compounds to cross-link cellular DNA. Proportion of DNA cross-linked by 500 μM *cis* Pt(II)diammine dichloride, 500 μM *trans* Pt(II)diammine dichloride, 500 μM *cis* Pt(IV)diammine tetrachloride and 500 μM *trans* Pt(IV)diammine tetrachloride following treatment of HeLa cells in suspension culture for 2 hr at 37°.

In Table 2 the concentrations of *cis* and *trans* Pt(II)diammine tetrachloride and *cis* and *trans* Pt(IV)diammine tetrachloride required to produce 10 per cent cross-linking of isolated and cellular DNA are recorded. It can be seen that the ratios of the *in vitro* to the *in vivo* doses required to produce an equal amount of DNA cross-linking are much larger for the platinum(II) compounds than for the platinum(IV) compounds, chiefly owing to the increased ability of the former to form interstrand cross-links in isolated DNA.

TABLE 2.

Compound	Dose required to produce 10% DNA cross-linking *in vitro* (A) (μM)	Dose required to produce 10% DNA cross-linking *in vivo* (B) (μM)	Ratio of A:B
Cis $Pt(II)(NH_3)_2Cl_2$	0·5	150	1:300
Trans $Pt(II)(NH_3)_2Cl_2$	1·0	1900	1:1900
Cis $Pt(IV)(NH_3)_2Cl_4$	33·0	420	1:12·7
Trans $Pt(IV)(NH_3)_2Cl_4$	67·0	570	1:8·5

DISCUSSION

Effects on cell survival. Cis Pt(IV)diammine tetrachloride has been shown to possess activity against the following experimental tumours: sarcoma 180,[3,4] leukaemia L1210[3] and reticulum cell sarcoma,[5] all of which are carried in the mouse, and lymphatic and myeloid leukaemias in rats.[6] It has also been shown to be immunosuppressive,[9] give rise to uraemia and kidney damage[6] and affect intestinal crypt cells[3] in treated animals. By contrast the *trans* isomer does not possess antitumour activity, nor cause toxic side effects.[6] These facts, together with the data presented in Fig. 1 concerning the effects of *cis* and *trans* Pt(IV)diammine tetrachloride on HeLa cell

survival, would suggest that the antitumour activity exhibited by *cis* Pt(IV)diammine tetrachloride is unlikely to be selective, but rather is the consequence of its general cytotoxic properties.

DNA cross-linking. In comparing the inactivation of bacteriophage T2 and ΦX 174 by nitrogen mustard and mustard gas, Loveless and Shields found that interstrand DNA cross-linking was the most important inactivating reaction where it was possible, and could be assumed to account at least partly for the considerably greater sensitivity of T2 bacteriophage to these agents. Nonetheless with nitrogen mustard, ΦX 174 owed some part of its inactivation to the bifunctional character of the agent indicating that intrastrand linkage could be more effective than two separate alkylations.[10] Studies on the inactivation of bacteriophages T7 and R17 by the platinum(II) compounds have shown that DNA interstrand cross-linking is unlikely to be the major cytotoxic lesion in these organisms because it is a relatively infrequent event, and it is therefore proposed that intrastrand linking of nucleic acid bases may be the most important inactivating reaction.[11]

The greater cytotoxicity of di(2-chloroethyl)sulphide compared to half sulphur mustard gas towards bacteria[12] and mammalian cells[13] was ascribed to its ability to form a cross-linked guanine product, di-(β-guanin-7-yl)ethyl sulphide. However the relative proportions of di-(β-guanin-7-yl)ethyl sulphide arising from DNA interstrand cross-linkage and DNA intrastrand cross-linkage were not determined.

The first condition to be satisfied if DNA cross-linking is to be an important cytotoxic reaction in the case of the inorganic platinum compounds, is that these compounds shall exert their cell killing effect by reacting directly with DNA. That this is probably the case has been indicated by observations concerning the effects of several platinum compounds on DNA, RNA and protein synthesis in human amnion AV_3 cells. Thus, treatment with low doses of active antitumour platinum compounds resulted only in inhibition of DNA synthesis, RNA and protein synthesis being unaffected.[2] We have confirmed these findings in HeLa cells.* and have shown that administration of doses of *cis* Pt(IV)diammine tetrachloride having measurable cell killing effects resulted in significant and persistent inhibition of DNA synthesis. Furthermore we have shown that *trans* Pt(IV)diammine tetrachloride could also inhibit DNA synthesis if treatment was carried out with doses high enough to cause measurable cell killing.† In view of the negligible effects of the inorganic platinum compounds on RNA and protein synthesis it would seem unlikely that inhibition of DNA synthesis is the result of RNA- or protein-mediated reactions, and therefore that the cytotoxic properties of the inorganic platinum compounds are most probably the result of their direct reaction with DNA.

If DNA interstrand cross-linking was the most important toxic lesion introduced into mammalian cells by the inorganic platinum compounds, it might be supposed that differences in the ability of a series of related compounds to form DNA interstrand cross-links could be correlated with differences in their cytotoxic properties. It is apparent however that the small difference in the ability of *cis* and *trans* Pt(IV)-diammine tetrachloride to cross-link isolated DNA is not great enough to account for the much larger difference in their cytotoxic properties as measured in the HeLa cell system. Neither is the small difference in the ability of the two compounds to

* J. M. Pascoe and J. J. Roberts, unpublished observations.
† J. M. Pascoe and J. J. Roberts, unpublished results.

cross-link cellular DNA (whether it results from similar extents of activation of *cis* and *trans* Pt(IV)diammine tetrachloride within whole cells to species capable of cross-linking DNA, or from some other mechanism) large enough to account for the difference in their abilities to kill HeLa cells. Therefore, in contrast to the situation obtaining in the case of the platinum(II) compounds,[7] it would appear that the ability to form interstrand cross-links in the DNA of whole HeLa cells bears little, if any, relationship to the cytotoxic properties of *cis* or *trans* Pt(IV)diammine tetrachloride. It is thus possible that variations in the abilities of these compounds to carry out some other bifunctional reaction, perhaps to form intrastrand DNA cross-links or DNA-protein cross-links, could account for their different cytotoxic properties.

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