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G_1 PHASE CHINESE HAMSTER V79-379A CELLS ARE INHERENTLY MORE SENSITIVE TO PLATINUM BOUND TO THEIR DNA THAN MID S PHASE OR ASYNCHRONOUSLY TREATED CELLS

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Abstract—The importance of expressing cell sensitivity in terms of extent of platinum bound to DNA is demonstrated using HeLa and Chinese hamster V79-379A cells. HeLa cells were found to be slightly more sensitive to *cis* platinum$_{[II]}$ diammine dichloride (*cis* $Pt_{[II]}$) for a given extent of platinum binding to DNA compared with Chinese hamster V79-379A cells, although the difference between the two cell lines was less than that indicated by dose–survival data. Chinese hamster cells in the G_1 phase of their cell cycles were more sensitive to treatment with *cis* $Pt_{[II]}$ than cells in other phases of the cell cycle or than cells in an asynchronous cell culture. This sensitivity of G_1 phase cells was not due to an increased uptake of platinum into the cells but rather reflected an inherent increase in sensitivity of these cells to a given binding of *cis* $Pt_{[II]}$ to their DNA. Chinese hamster cells were inherently more sensitive to the drug when they were treated in a stationary growth phase followed by dilution as compared with treatment in an exponential growth phase.

Cis platinum$_{[II]}$ diammine dichloride (*cis* $Pt_{[II]}$) is currently being used for the treatment of a number of human tumours [1–3]. There is extensive evidence, accumulated over a number of years, to indicate that the cytotoxic properties of *cis* $Pt_{[II]}$ result from its ability to bind to DNA and modify this as a template for normal DNA replication [4–6]. The extent of reaction of *cis* $Pt_{[II]}$ which occurs with cellular DNA at a measured level of cell killing thus constitutes an indication of the inherent sensitivity of a cell to the drug. Hence the levels of reaction at equitoxic doses of the drug to various cell types could therefore reflect the different abilities of cells to repair or withstand damage to their genome, either by excising lesions from their DNA prior to DNA replication, or by circumventing lesions in DNA during DNA synthesis by some form of replication repair. Such differences between cells could account for the selective action of platinum compounds on certain experimental and human tumours.

In this paper we report on the relative sensitivities of HeLa and Chinese hamster cells in culture to various doses of *cis* $Pt_{[II]}$ and demonstrate the importance of expressing comparative sensitivities in terms of extent of Pt bound to the DNA. Chinese hamster cells are shown to be more sensitive to *cis* $Pt_{[II]}$ treatment in the G_1 phase compared to other phases of the cell cycle. This finding was confirmed when the extent of reaction of Pt with the DNA in G_1 phase cells was compared with that in mid S phase cells. These comparative studies further emphasize that cell types or cells in various physiological states can differ in their uptake of, or sensitivity to, the platinum drug.

MATERIALS AND METHODS

Chemicals. Cis $Pt_{[II]}$ diammine dichloride was a gift from Johnson Matthey Research Centre; [6-^{3}H]Thymidine, 25 Ci/m-mole, was obtained from The Radiochemical Centre, Amersham, U.K.

Cell culture. The conditions for growing Chinese hamster V79-379A(V79) and HeLa S3 cells have been described [7]. Synchronously growing cell cultures were obtained either by the mitotic harvest technique of Robbins and Marcus [8] or by a stationary phase cell dilution technique [9]. This latter method was used when large numbers of synchronised cells were required. Although it produces a less synchronised population of cells than is obtained by mitotic harvest, it was adequate for the present study.

Treatment of cells with *cis* $Pt_{[II]}$, estimation of colony forming ability [7] and isolation of DNA [10, 11] were as described previously.

Estimation of platinum. The platinum content of hydrolysates was measured using a Perkin Elmer (model 306) Atomic Absorption spectrometer as previously described [11].

Estimation of the rate of DNA synthesis in synchronously growing cells. Aliquots of 1 ml were removed from synchronously growing cultures and incubated in an equal volume of spinner culture medium containing 1 μCi/ml [^{3}H]thymidine for 20 min at 37°. Incorporation of radioactivity was stopped by the addition of excess ice-cold saline, the cells harvested on Whatman GF/C glass fibre filters, washed with 5% trichloroacetic acid and the radioactivity determined as previously described [12].

RESULTS

Relationship between exogenous dose of cis $Pt_{[II]}$ *and survival.* Survival data for both Chinese hamster V79 and HeLa cells in response to *cis* $Pt_{[II]}$ treatment for 1 hr are summarised in Fig. 1. HeLa cells apparently showed a greater sensitivity to a given dose of *cis* $Pt_{[II]}$

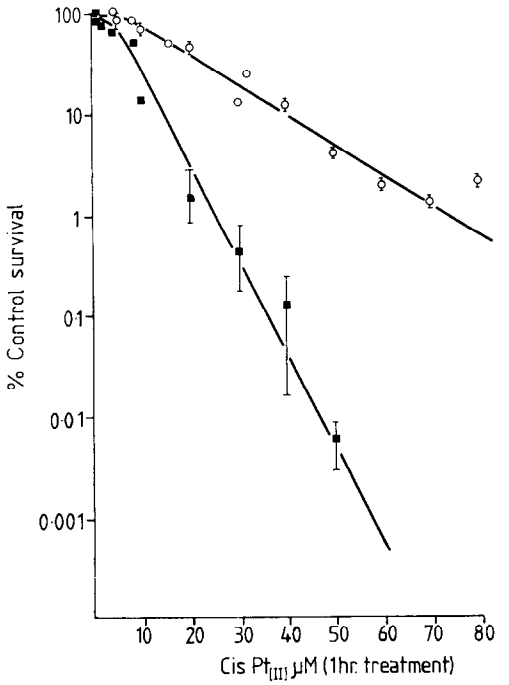

Fig. 1. The relative sensitivities of HeLa (■) and Chinese hamster V79 (○) cells to a 1-hr treatment of *cis* $Pt_{[III]}$, measured as survival after a given dose. The vertical bars illustrate the standard error of the mean.

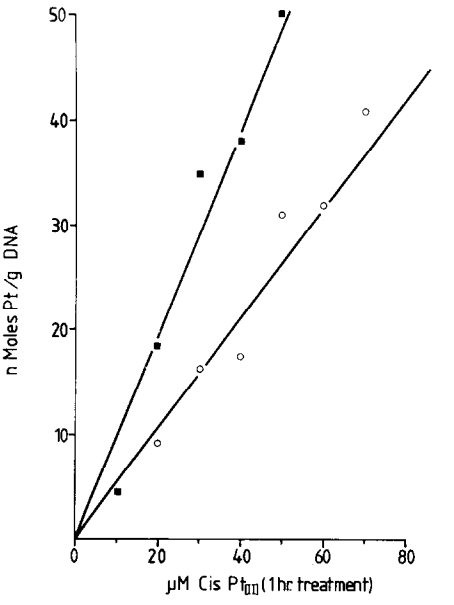

Fig. 2. The relative extent of reaction of *cis* $Pt_{[III]}$ to the DNA of HeLa (■) and Chinese hamster V79 (○) cells as a function of dose. The DNA of cells treated with *cis* $Pt_{[III]}$ and assayed for survival was isolated, hydrolysed in 1N HCl and the platinum content was determined by atomic absorption spectroscopy.

than Chinese hamster V79 cells. The D_0 dose (dose required to reduce survival from a fraction f to $0.37f$ on the exponential part of the survival curve) was 15 μM for Chinese hamster cells compared with 4.6 μM for HeLa cells. Thus HeLa cells were 3.3 times more sensitive to *cis* $Pt_{[III]}$ than V79 cells on a dose vs survival basis.

Relationship between dose of cis $Pt_{[III]}$ *and binding of platinum to DNA.* To establish whether the difference between the two cell lines in their toxic response to *cis* $Pt_{[III]}$ reflected a real difference in binding of platinum to DNA, both cell lines were treated with various concentrations of *cis* $Pt_{[III]}$ for 1 hr and the platinum content of isolated DNA was determined by atomic absorption spectroscopy. Figure 2 shows the relative binding of *cis* $Pt_{[III]}$ to the DNA of HeLa and Chinese hamster V79 cells as a function of *cis* $Pt_{[III]}$ dose. The extent of platinum reaction with the DNA of both cell lines increased linearly with increasing *cis* $Pt_{[III]}$ treatment concentrations. However, HeLa cells appeared to bind 1.85 times more platinum for a given dose of *cis* $Pt_{[III]}$ than Chinese hamster V79 cells. Our results therefore indicate that HeLa cells are more permeable to *cis* $Pt_{[III]}$ than are Chinese hamster V79 cells. Since *cis* $Pt_{[III]}$ is thought to diffuse passively through the membrane the observed difference is presumably a result of differences in either the cell wall composition or topography between the two cell lines.

Relationship between binding of platinum to DNA and survival. For each *cis* $Pt_{[III]}$ treatment of the two cell lines, a colony forming assay was used to determine the toxicity of the *cis* $Pt_{[III]}$ in the same population of cells used for DNA binding determinations. The relationship between the extent of platinum reaction with DNA and the effect on cell survival is illustrated in Fig. 3. Chinese hamster V79 cells have a B_0 (the binding required to reduce survival from a fractional f to $0.37f$ on the exponential part of the curve) of 8.5 nmoles/g DNA compared with 5.5 nmoles/g DNA for HeLa cells. On the basis of these data HeLa cells are more sensitive to

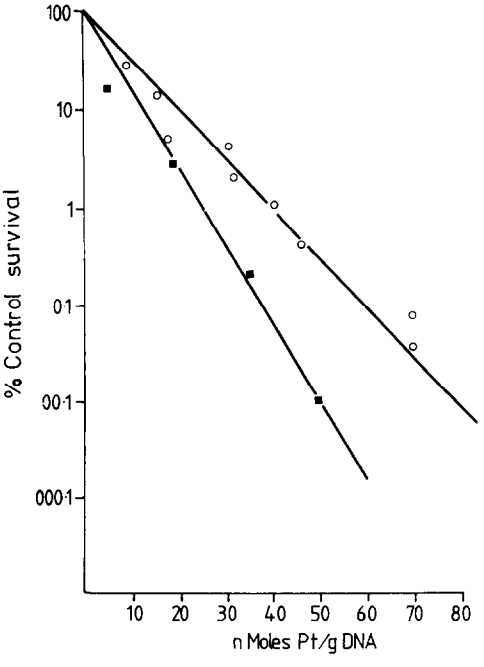

Fig. 3. The relative sensitivities of HeLa (■) and Chinese hamster V79 (○) cells to *cis* $Pt_{[III]}$ expressed as a function of platinum bound to DNA.

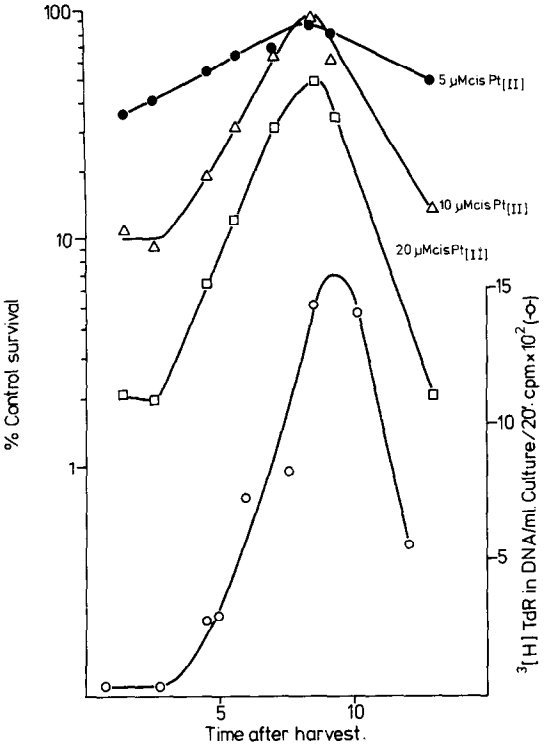

Fig. 4. The variation of the toxicity of *cis* Pt_{III} in Chinese hamster V79 cells when treated at various times during the cell cycle. Aliquots of cells synchronised by the mitotic harvest technique were removed at various times after harvest and treated with 5, 10 and 20 μM *cis* Pt_{III} for 1 h. The per cent control survival, estimated by colony forming ability, was plotted against time of treatment after harvesting. The rate of DNA synthesis (O—O) was determined to indicate the mid S and G_1 phases of the cell cycle.

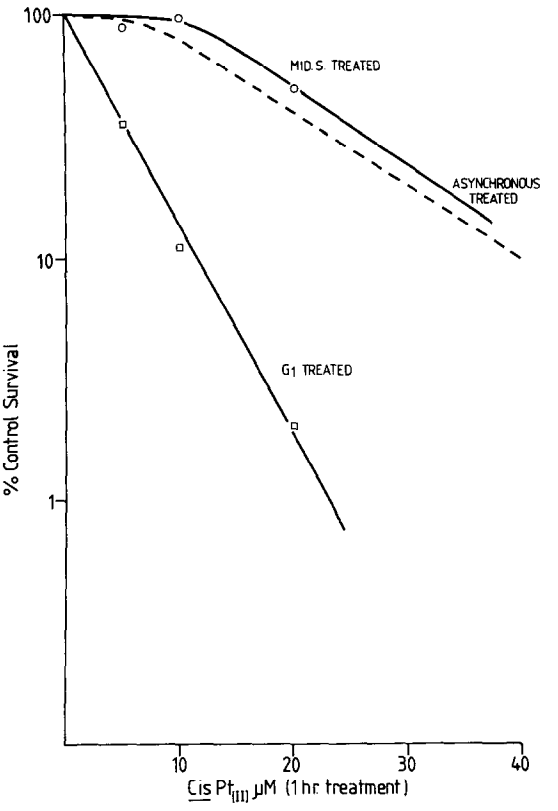

Fig. 5. The survival data for mid S phase (O) and G_1 phase (□) treated Chinese hamster V79 cells illustrated in Fig. 4 plotted as a function of *cis* Pt_{III} dose. The discontinuous line represents the survival obtained using an asynchronous culture.

platinum damage by a factor of 1.55 compared to Chinese hamster cells, approx. half the value calculated from the dose–survival data.

Cell cycle variations in survival of cis Pt_{III}*-treated Chinese hamster cells.* Variations were found in the toxic effect of a 1-hr treatment with *cis* Pt_{III} when added to cells in different phases of the Chinese hamster cell cycle (mitotically harvested cells) (Fig. 4). The rate of DNA synthesis was monitored during the experiment and is illustrated in the lower section of Fig. 4 to provide an indication of the progression of the cell cycle. Generally it can be seen that G_1 phase cells exhibited maximum, and mid S phase cells minimum sensitivity to *cis* Pt_{III}. Thus treatment of Chinese hamster cells with 20 μM *cis* Pt_{III} for 1 hr reduced the survival of G_1 phase cells to 2 per cent and of mid S phase cells to only 35 per cent of control cells. Similar variations were observed in synchronous Chinese hamster cells treated with doses of 5 and 10 μM *cis* Pt_{III}. Plots of log survival against concentrations of *cis* Pt_{III} gave D_0 values of 5 and 14 μM for G_1 and mid S phase treated Chinese hamster cells respectively (Fig. 5). The broken line in Fig. 5 indicates the sensitivity of cells treated in asynchronous culture.

Reaction of cis Pt_{III} *wih the DNA of* G_1 *and S phase cells.* To answer the question 'Are cells in G_1 phase more sensitive than cells in other phases of the cell cycle because their uptake of *cis* Pt_{III} is greater for a given dose?', we undertook an investigation into the binding and survival of G_1 and mid S phase Chinese hamster cells following treatment with *cis* Pt_{III}. The large numbers of cells required for this study precluded the use of the mitotic harvest technique for obtaining synchronous cells. Instead we used a stationary phase cell dilution technique. A stirred cell suspension was grown to stationary phase (approx. 2×10^6 cells/ml) and maintained in this condition for 2 days. A 10-fold dilution of these cells using fresh medium resulted in a largely synchronous population of cells being produced (Fig. 6B). Aliquots were removed from this cell population at times T_1 and T_2 after dilution of the stationary culture (Fig. 6B) and were treated with *cis* Pt_{III} for 1 hr. The aliquots were divided into two portions, one for estimation of cell survival and the other, containing 2×10^8 cells, for determination of the extent of platinum bound to DNA. The binding of Pt to DNA vs survival data obtained for late G_1 and aproximately mid S phase treated Chinese hamster cells are shown in Fig. 6A. The G_1 phase treated Chinese hamster V79 cells had a B_0 dose of 3 nmoles Pt/g DNA compared to 6.5 nmoles Pt/g DNA for mid S phase treated cells. Thus it is clear that for a given binding of Pt to DNA, G_1 phase Chinese hamster cells are more sensitive than mid S cells or asynchronously growing cells.

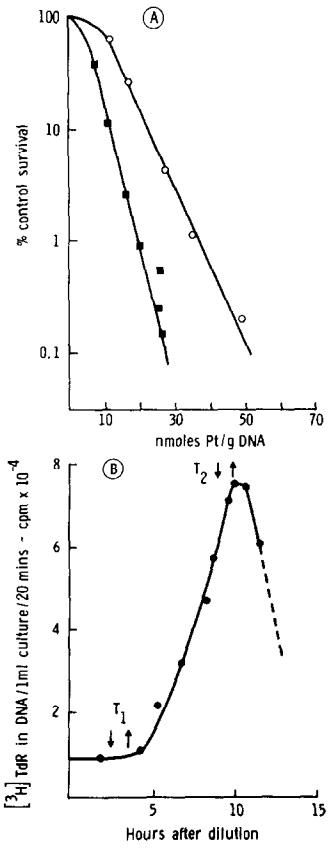

Fig. 6. The relative extent of reaction of *cis* $Pt_{(III)}$ in mid S phase and G_1 phase treated Chinese hamster V79 cells compared to survival. (A) The extent of reaction of Pt to DNA vs survival in mid S (○) and G_1 phase treated (■) cells. (B) The rate of DNA synthesis measured during a 20 min pulse at various times after dilution of a stationary phase Chinese hamster V79 culture. Samples of the culture were treated for 1 h at T_1 (G_1 phase) and T_2 (mid S phase) and the extent of Pt binding to DNA and survival estimated.

DISCUSSION

Relative sensitivities of HeLa and Chinese hamster cells to cis $Pt_{(III)}$. The sensitivities of cells to the DNA damaging effect of platinum compounds can be quantitated by the slopes (B_0) of the exponential part of the curves relating cell survival after treatment with a given dose of platinum drug to the extent to reaction which occurs with cellular DNA after such treatment. A comparison of data derived in this way indicated that HeLa cells were more sensitive to *cis* $Pt_{(III)}$ than Chinese hamster cells, the slopes of the respective lines being 1:1.55. Previous attempts [13] to obtain such values had not compared the binding to cellular DNA in the same culture that was used to measure cell survival and were accordingly less accurate than those reported here. The results further show that the sensitivity to *cis* $Pt_{(III)}$ of Chinese hamster cells (B_0 8.5 nmoles/g DNA) is comparable to that of human foetal lung cells (B_0 7.4 nmoles/g DNA [11]).

A previous comparison of the response of Chinese hamster V79 cells and HeLa cells to the methylating agents, *N*-methyl-*N*-nitrosourea (MNU) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and the difunctional alkylating agent sulphur mustard (SM) indicated that whereas HeLa cells appeared to be considerably more sensitive than Chinese hamster cells to the methylating agents there was little difference in their sensitivity to SM. It therefore follows that the relative responses of these two cell lines to damage introduced by *cis* $Pt_{(III)}$ more closely resemble those to SM than to the methylating agents and may reflect the bifunctional nature of the damage introduced by both SM and *cis* $Pt_{(III)}$. In addition these findings are consistent with the view that DNA is the cytotoxic target for both the difunctional alkylating agents and *cis* $Pt_{(III)}$.

HeLa and Chinese hamster cells have been shown to respond differently to *cis* $Pt_{(III)}$ induced damage with respect to effects on the rates of DNA synthesis in G_1 phase treated synchronously growing cells [14, 15] and in respect of their sensitivities to post-treatment incubation in the presence of caffeine [14–16]. However, these two cell lines have been shown to display comparable DNA excision repair capacities, as indicated by their rates of removal of 7-bromomethylbenz(a)anthracene (7-BMBA) bound products from DNA [17] or the extent of *N*-methyl-*N*-nitrosourea-induced repair synthesis [18]. It has therefore been concluded that the above differences between the two cell lines could reflect a difference in some DNA repair pathway other than excision repair. Thus the mechanism by which cells can replicate their DNA on a template containing unexcised lesions ('replication repair') can be inhibited by caffeine in Chinese hamster cells [16, 19, 20] but not in HeLa cells [15, 20]. The caffeine-insensitive replication repair of HeLa cells may thus be less efficient than the caffeine-sensitive mechanism of Chinese hamster cells and account for the observed small difference in sensitivity of the two cell lines to *cis* $Pt_{(III)}$ as reported here.

Cell cycle fluctuations in sensitivity to cis $Pt_{(III)}$. The greater sensitivity of the G_1 phase compared to other phases of the cell cycle to treatment with *cis* $Pt_{(III)}$ for Chinese hamster cells is reminiscent of the greater sensitivity of G_1 phase cultured human lymphoma cells compared to mid S phase treated cells as reported by Drewinko *et al.*[21]. However, the differences in sensitivity throughout the cell cycle were much less marked in the case of the human lymphoma cells than those reported here for Chinese hamster cells. By contrast, Szumiel and Nias[22] did not detect any differences in sensitivity throughout the cell cycle of Chinese hamster ovary cells following treatment with *cis* dichlorobis(cyclopentylamine)platinum$_{(III)}$. Comparison of our data with numerous other studies on fluctuations in sensitivity during the cell cycle following treatment of cells with alkylating agents (MMS [23,24], HN2 [25,26], SM [26–28], uracil mustard [26], phenylalanine mustard [29], chlorambucil [29]), 4-nitroquinoline-1-oxide[30] and X-rays [27] there seems to be general agreement that part or all of the G_1 phase of the cell cycle is relatively more sensitive than other phases. In general the alkylating agents were found to be least toxic to cells in the late S phase–G_2 phase of the cell cycle. By contrast cells in mid S phase were least toxically affected by *cis* $Pt_{(III)}$.

Why then is the G_1 phase of cell cycle the most sensitive to *cis* $Pt_{(III)}$ treatment? Certainly there is not an increased uptake of *cis* $Pt_{(III)}$ into G_1 phase cells compared to mid S phase cells. G_1 phase cells appear to be

inherently more sensitive to platinum induced damage to their DNA than other phases of the cell cycle.

Two possible explanations for this difference occur to us. Replication of a whole genome immediately after platinum treatment would be expected to result in more damage (unligated DNA)[19,31] at the end of the cell cycle compared to mid S phase treatment when only part of the DNA is replicated immediately after treatment. Although excision repair is a relatively slow process [17,32] a proportion of the platinum damage should have been removed before the start of the next round of DNA replication. An alternative possibility is that base sequences in DNA replicated at the start of the S phase contain a relatively greater proportion of platinum than those replicated in late S phase. Two lines of evidence are consistent with this proposition. *cis* $Pt_{(II)}$ has been shown to react more readily with guanine than with the other bases in DNA [33–35]. In addition Tobia *et al.* [36] have demonstrated in both mouse str L cells and HeLa cells that DNA synthesised in the early period of the S phase had an average guanine plus cytosine (G + C) content of 43.6 per cent and that DNA synthesised late in the S phase had an average G + C content of 38.7 per cent. The average G + C content was reported as 40.1 per cent. The combination of these two factors could result in a higher concentration of platinum being present on that DNA which is replicated early in the S phase than on the DNA which is replicated in late S phase. Treatment in mid S phase would therefore allow a period during which platinum could be removed by excision repair from early S phase base sequences before their replication in the next cell cycle.

Sensitivity of stationary phase cells to cis $Pt_{(II)}$. Chinese hamster cells in stationary phase or G_1 phase were more sensitive to treatment with *cis* $Pt_{(II)}$ than cells growing exponentially. Thus for a given extent of binding of $Pt_{(II)}$ to the DNA G_1 phase cells demonstated a greater sensitivity compared to mid S phase cells. The increased sensitivity of stationary phase cells to dimethylsulphate (DMS) had been shown previously not to be the result of any increased uptake of drug [9] nor to be associated with any decreased ability to repair DMS-induced DNA strand breaks [9]. On the other hand, decreased excision repair capacity is a likely explanation of the present findings since stationary phase cells do show a decreased ability, as compared with exponentially growing cells, to excise *cis* $Pt_{(II)}$ bound adducts to their DNA [32]. Although cells not undergoing DNA replication (G_1 and stationary) appear to be the most sensitive to *cis* $Pt_{(II)}$ treatment, there is considerable evidence to support the notion that DNA synthesis is required to express cellular toxicity in cells treated with *cis* $Pt_{(II)}$ [5]. Thus cells that were maintained in stationary phase for 3 days after treatment with *cis* $Pt_{(II)}$ prior to dilution into fresh medium for determination of colony forming ability show a 2 decade increase in survival compared to cells diluted immediately after treatment [32]. Such recovery of colony forming ability can be ascribed to the loss of DNA bound platinum adducts during this time period.

The findings reported in this paper suggest a number of explanations for differences in the cytotoxic response of various tumours or tissues to the antitumour platinum compounds. Apart from possible differences in the uptake of the agents into different cell types, the ultimate killing effect will also depend on the cell cycle parameters of the treated cells, cells with a longer relative G_1 phase probably being more sensitive to the drug. In addition it can be seen how the proportion of tumour cells moving into and out of a G_0 phase of the cells cycle would affect its response to this agent.

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