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PLATINUM COMPLEXES AS RADIOSENSITIZERS OF HYPOXIC MAMMALIAN CELLS

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Summary.—*Cis*-dichlorodiammineplatinum(II), or *cis*-PDD, has recently been shown to be a potent radiosensitizer of bacteria, particularly under conditions of acute hypoxia. This study extends this observation to include the radiosensitization of mammalian cells (V-79) by low concentrations of *cis*-PDD, *cis*-dichlorobis(aziridine)platinum(II), and the *trans*-isomer of PDD as measured from survival curve analysis. This radiosensitization was obtained at concentrations of 10 μM, 60 μM, and 100 μM for the *cis*-PDD, aziridine-platinum, and *trans*-isomer respectively. The corresponding drug toxicity survival levels were 8, 40 and 75%. Dose modification factors of around 1·3 to 1·4 were observed.

EXPERIMENTS by Richmond and Powers (1976) demonstrated that the chemotherapeutic agent *cis*-dichlorodiammineplatinum(II), or *cis*-PDD, is a potent radiation sensitizer of the metabolically inactive *B. megaterium* spore. This sensitization is approximately twice as great in the absence of oxygen as in the presence of oxygen. Richmond, Zimbrick and Hykes (1977) have also shown that *cis*-PDD is an efficient radiation sensitizer of the metabolically active *E. coli* bacterium, especially in the absence of oxygen. Both these groups of authors have suggested that their results might be extended to mammalian cells and that these results have significant clinical implications.

Preliminary experiments by Deen and Richmond (unpublished results) suggested that radiosensitization of hypoxic V-79 Chinese hamster cells was demonstrable using concentrations of less than 10 μM *cis*-PDD. At approximately the same time, Szumiel and Nias (1976) and Chadwick *et al.* (1976) reported that pre-treatment with another platinum coordination complex, *cis*-dichlorobis(cyclopentylamine)-platinum(II), or PAD, reduced the D_0 value of the subsequent radiation survival curves of well-oxygenated CHO cells. More recently these same authors (Nias and Szumiel, 1977) have reported a similar synergistic interaction of the platinum complex *cis*-dichlorobis(isopropylamine)-*trans*-dihydroxyplatinum(IV), or CHIP, with ionizing radiation.

Wodinsky *et al.* (1974) have reported potentiation between *cis*-PDD and radiation on prolonging the life span of mice previously inoculated with P388 lymphocytic leukaemia cells. We have also recently reported therapeutic potentiation when a mouse mammary adenocarcinoma or an intracerebral rat brain tumour are treated with *cis*-PDD before irradiation (Douple, Richmond and Logan, 1977).

As a result of these characterizations of *cis*-PDD as a potential radiation sensitizer of bacteria and considering the importance of drug–radiation interactions for cancer treatment, a study was undertaken to characterize the effects of the combined treatment of platinum complexes and radiation on well-oxygenated and hypoxic V-79 mammalian cells. The 3 platinum complexes tested were *cis*-PDD,

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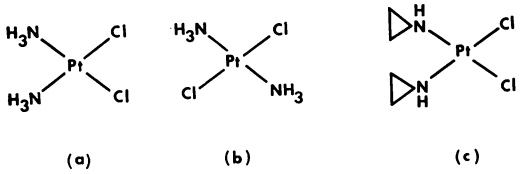

FIG. 1.—Chemical structure of platinum complexes: (a) *cis*-dichlorodiammine-platinum(II) (b) *trans*-dichlorodiam-mineplatinum(II) (c) *cis*-dichlorobis(azirí-dine)platinum(II).

the *trans*-isomer of PDD and *cis*-dichloro-bis(aziridine)platinum(II). These complexes are known to represent a wide range of therapeutic indices and toxicities *in vivo* in a large family of chemotherapeutic agents (Connors *et al.*, 1972). The structures of these coordination complexes are shown in Fig. 1.

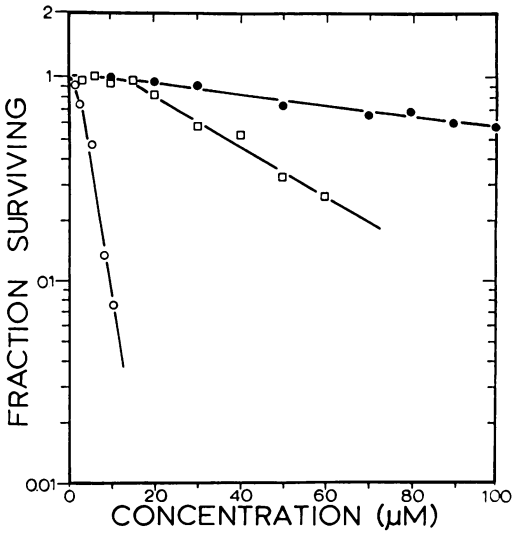

FIG. 2.—Drug toxicity survival curves: *cis*-PDD (open circles); aziridine–platinum (squares); and *trans*-PDD (closed circles).

MATERIALS AND METHODS

Chinese hamster lung fibroblasts, V-79, were treated as unfed, confluent, plateau-phase monolayers (6-8 days old) in 60mm diameter plastic (Falcon) or glass dishes. At this time the dishes contain approximately 4×10^6 cells in 5 ml of sodium bicarbonate buffered BME (with Hanks' Salt Base) supplemented with heat-inactivated foetal bovine serum (15%) and 1 ml of antibiotic mixture per 100 ml of BME. All media constituents were obtained from Associated Biomedic Systems, Inc. The monolayer cultures were grown as described previously (Douple, 1976).

The *cis*-PDD and the aziridine–platinum complex were obtained by courtesy of the Drug Development Branch of the National Cancer Institute and the National Institutes of Health. The *trans*-PDD was kindly provided by Dr Henry J. Peresie. Stock solutions of the platinum complexes were prepared fresh in sodium bicarbonate-buffered Hanks' Balanced Salt Solution (Microbiological Associates). The drugs were added to the dishes and the cells were incubated at 37°C for 2 h before trypsinization and subculture was followed by conventional colony-forming assay methods.

Radiation survival curves were measured for both well-oxygenated and hypoxic cells. Hypoxia was obtained by approximating the procedure of Koch and Kruuv (1971). The glass dishes were placed into aluminum chambers which were attached to a degassing manifold. Removal of air from the chambers by pumping, backfilling with purified nitrogen, and returning of the chambers to the incubator was repeated 4 times. The cells were irradiated with an Eldorado-6 (Atomic Energy of Canada Limited) cobalt source with a dose rate of approximately 265 rad/min. Drug-treated cells were irradiated so that the irradiation was completed 2 h after addition of the drug.

RESULTS

The response of the V-79 cells to 2-h exposures of various concentrations of the 3 platinum complexes is illustrated in Fig. 2. Differences in the shapes of the toxicity curves are apparent among the very toxic *cis*-PDD, the moderately toxic aziridine-platinum, and the relatively non-toxic *trans*-isomer of PDD.

The survival data for the combined drug-radiation treatments are shown in Figs. 3–5, illustrating results from 10 μM *cis*-PDD, 60 μM aziridine-platinum and

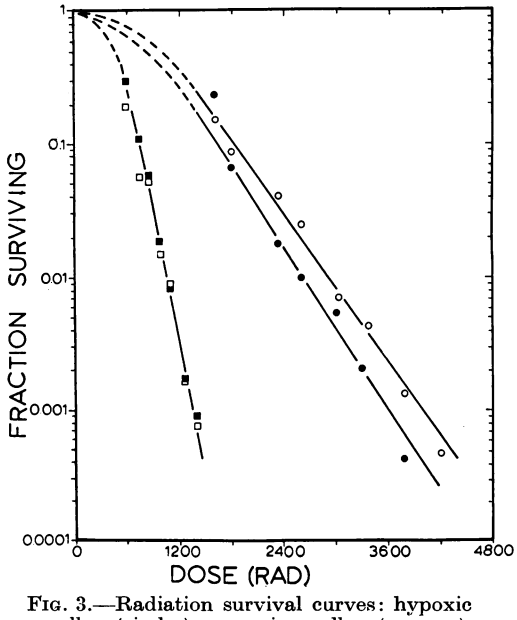

FIG. 3.—Radiation survival curves: hypoxic cells (circles); euoxic cells (squares). Closed symbols received 2 h pretreatment with 10 μM *cis*-PDD.

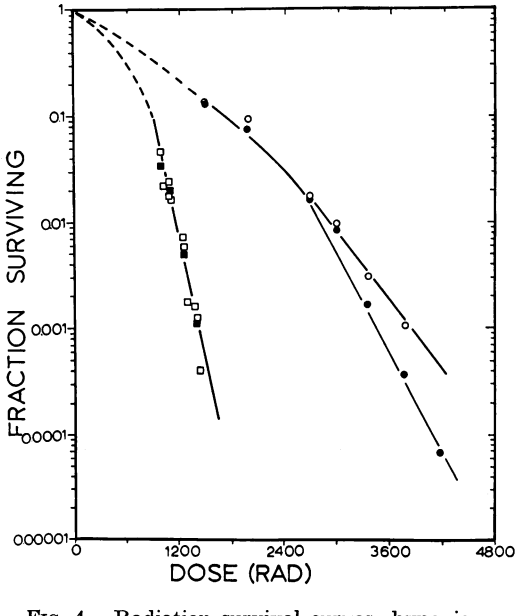

FIG. 4.—Radiation survival curves: hypoxic cells (circles); euoxic cells (squares). Closed symbols received 2 h pretreatment with 60 μM aziridine–platinum.

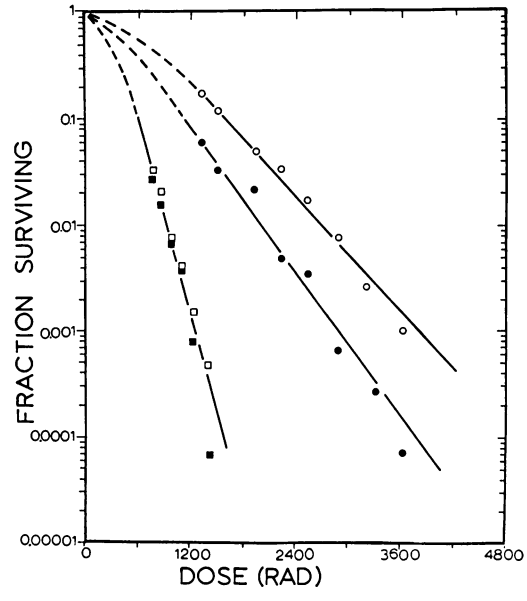

FIG. 5.—Radiation survival curves: hypoxic cells (circles); euoxic cells (squares). Closed symbols received 2 h pretreatment with 100 μM *trans*-PDD.

100 μM *trans*-PDD. The data have been normalized to survival after drug treatment alone. There is a difference between the final slopes in each pair of hypoxic curves for the 3 complexes, but at different drug concentrations and different levels of drug toxicity. Radiosensitization was not observed at concentrations of less than 10 μM PDD or 30 μM aziridine–platinum. The greatest effect was observed with *trans*-PDD, primarily because of sensitization at lower radiation doses. However, the dose modification factor (DMF) of approximately 1·3 is similar to that calculated for *cis*-PDD or aziridine–platinum.

DISCUSSION

These results with mammalian cells suggest that the 3 platinum complexes tested are hypoxic radiosensitizers of variable effectiveness. Sensitization by *cis*-PDD is somewhat anomalous in that this effect is observed in the high-dose region of the survival curve and the extrapolation

number is changed. This high-dose effect may reflect accumulation of damage to some critical threshold, perhaps related to secondary reactions of platinum intermediates (*e.g.*, Pt(I)), or accumulation of active platinum radiolysis products.

We presume that both *cis* and *trans*-PDD should sensitize equally well within the framework of generally cited sensitizer mechanisms. Differences in membrane permeability or intracellular distribution between *cis*- and *trans*-PDD may, of course, affect differences in the sensitization by these complexes. Nonetheless, it is of interest that *trans*-PDD at 100 μM does sensitize hypoxic cells in the normal way. That is, the extrapolation number is the same from hypoxic survival curves obtained in the presence and absence of *trans*-PDD, indicating that sensitization is achieved uniformly over both the low-dose and high-dose regions. One might expect, then, that *cis*-PDD at 100 μM would provide similar sensitization, perhaps in addition to its anomalous high-dose effect. Unfortunately, the toxicity of *cis*-PDD establishes 10 μM as a limiting concentration in these *in vitro* experiments, so that this speculation cannot be tested directly. A concentration of 10 μM *cis*-PDD is not effective in sensitizing bacterial spores (Richmond and Powers, 1976) or vegetative cells (Richmond, Zimbrick and Hykes, 1977), but 50 μM is effective. The marginal effect of *cis*-PDD at 10 μM and the more fully developed effect of *trans*-PDD at 100 μM may therfore be correlated to these bacterial models.

The platinum complexes represent a new array of cancer chemotherapeutic agents, including several drugs with larger therapeutic indices than *cis*-PDD (Rosenberg, 1973; Cleare, 1977). Other platinum complexes might react differently when combined with ionizing radiation, as suggested by the report of Nias and Szumiel (1977), which indicates that PAD and CHIP interact with radiation to produce a significant enhancement ratio in well-oxygenated cells. Combined treatment of animal tumour models with *cis*-PDD and radiation has already provided encouraging results (Douple, Richmond and Logan, 1977) using a dose of *cis*-PDD that approximates *in vivo* the 10 μM found to be somewhat effective in these *in vitro* studies. It is likely that employing other platinum antitumour drugs of higher therapeutic indices (usually less toxic to normal tissue than *cis*-PDD) will permit higher *in vivo* concentrations and a corresponding increase in radiation sensitization of tumour cells. Using the *trans*-PDD results as an approximation for effective platinum(II) complexes, an increase of *in vivo* platinum concentration to 100 μM might be expected to add a significant component of radiation sensitization to the combined treatment advantages already established in the case of *cis*-PDD.

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