The Use of Platinum Chemotherapy to Potentiate Radiotherapy

PRECLINICAL RESULTS ENCOURAGE CLINICAL TRIALS

By E. B. Douple
Dartmouth Medical School, Hanover, New Hampshire, U.S.A.

During the past decade the parent complex *cis*-dichlorodiammineplatinum(II) [*cis*-DDP or Cisplatin] has become an important clinical chemotherapeutic agent for treating a variety of cancers. Clinical testing of second generation platinum complexes has also been encouraging. At the same time, results of pre-clinical studies have suggested an additional role for *cis*-DDP; potentiation of the cell killing effects of radiation therapy producing more cell killing than expected on the basis of the two agents acting in an additive fashion. Furthermore, there is reason to believe that the second generation platinum complexes such as diammine(1,1-cyclobutanedicarboxylato)platinum(II) [JM8 or Carboplatin] and *cis*-dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine)platinum(IV) [JM9 or Iproplatin] might be more effective radiation potentiators. It may be beneficial to consider a dual role for platinum complexes; tumouricidal activity and selective radiation sensitisation. A rationale based on results of pre-clinical studies has encouraged the design of clinical trials now underway to investigate this potential.

Importance of Radiosensitisation

Radiation therapy is known to be less effective when cells are deficient in oxygen (hypoxic or anoxic). Radiation induced cell killing (inhibition of mitotic activity) results in a steeper killing curve if oxygen is present, as illustrated in Figure 1. The cell killing factors (D_0 values, the inverses of the slopes of the exponential regions of the survival curves) for the aerated and anoxic curves differ by approximately 3.0, and this enhancement due to the presence of oxygen is called the oxygen enhancement ratio (OER). Tumours are expected to contain a significant population of viable hypoxic cells, since oxygen is consumed before it penetrates distances greater than about 100 microns from blood vessels, as illustrated in Figure 2.

A major radiobiology research goal has been to overcome this radioresistance of tumour cells which might account for some of the cancer treatment failures. One approach has been to identify chemicals which are oxidising agents that operate as radiosensitisers of hypoxic cells through free radical events that occur during irradiation. A therapeutic gain might be expected if hypoxic cells in tumours are radiosensitised without any enhancing effect on aerated cells, assuming that most normal tissues are well oxygenated. Classes of electron affinic agents such as the nitroimidazoles were identified, and some of these compounds such as misonidazole were introduced into clinical trials for testing as radiosensitisers. Although a significant reduction in the OER by misonidazole was demonstrated in cultured cells in petri dishes and in some transplanted animal tumours, misonidazole proved to be neurotoxic. Plasma levels required for radiosensitisation could not be achieved in patients. However, second generation radiosensitisers have been developed and are currently undergoing clinical testing (1).

The Discovery that *cis*-DDP is a Radiosensitiser

In 1976 Richmond and Powers reported that *cis*-DDP enhanced the radiation-induced killing of hypoxic bacterial spores at relatively low concentrations above 10 micromolar (2). The results of this study suggested that the radiation

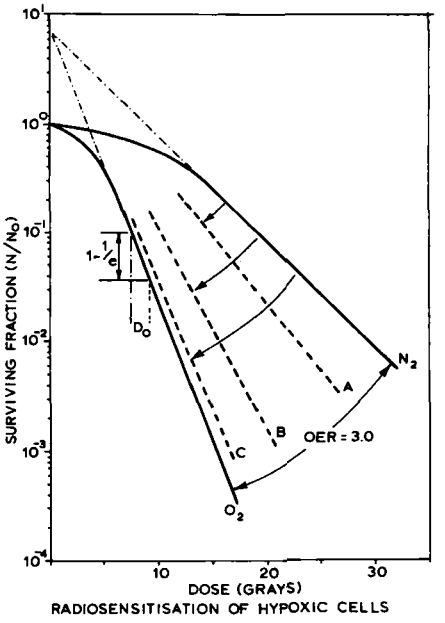

Fig. 1 Hypothetical radiation-induced killing of cultured mammalian cells irradiated under hypoxia (N_2; $D_0 = 4.5$ Gy) or aeration (O_2; $D_0 = 1.5$ Gy) with an OER of 4.5/1.5 or 3.0. Curve A represents the shift in survival of hypoxic cells produced by an agent with ER = 1.3 as reported by some investigators for *cis*-DDP (Ref. 7). Curve B is the effect of ER = 1.9 as reported for the second generation clinical complex Iproplatin under certain conditions (Ref. 35). Curve C is an ER = 2.4 reported for FLAP, a platinum complex containing a radiosensitiser moiety; this effect was found in some laboratories (Ref. 37) but not in all studies (Ref. 38). The radiosensitiser undergoing clinical trials, misonidazole, produces an ER greater than 2.4 at concentrations of about 10 mM (Ref. 1). The hypoxic cell sensitiser is not expected to shift the O_2 curve, that is under aerated conditions. Construction of these curves corrects for cell kill from the chemicals alone, reflecting enhancement of the radiation-induced cell kill. These curves have assumed no effects on curve shape at low doses of irradiation

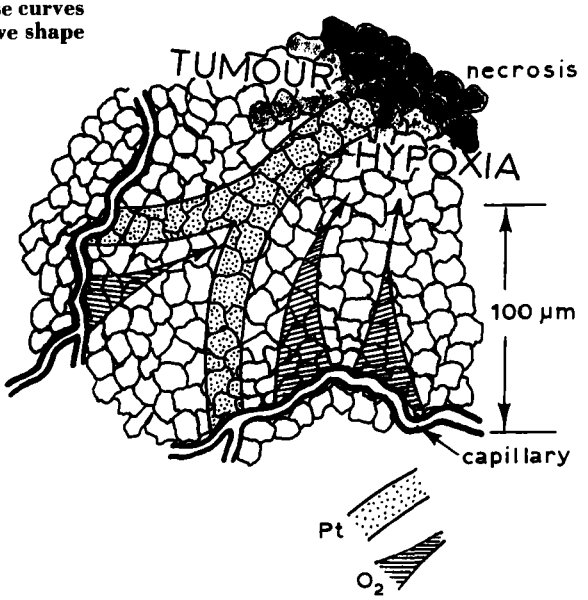

Fig. 2 Cells located at distances greater than approximately 100 microns from the capillaries are expected to contain populations of viable but hypoxic cells since O_2 is consumed as it diffuses towards this population. It is hoped that platinum complexes (Pt) will have less steep gradients and will penetrate into the region of tumour hypoxia. This will be a necessary requirement if platinum complexes are to serve as radiosensitisers of hypoxic cells

sensitisation included a free radical component. The interaction between this metal complex and radiation was not necessarily a simple mechanism since there was some smaller potentiation of oxygenated spores. This observation has been extended to include vegetative bacteria (3) and cultured mammalian cells (4).

Richmond and co-workers have probed the mechanisms of the interactions in bacteria, and

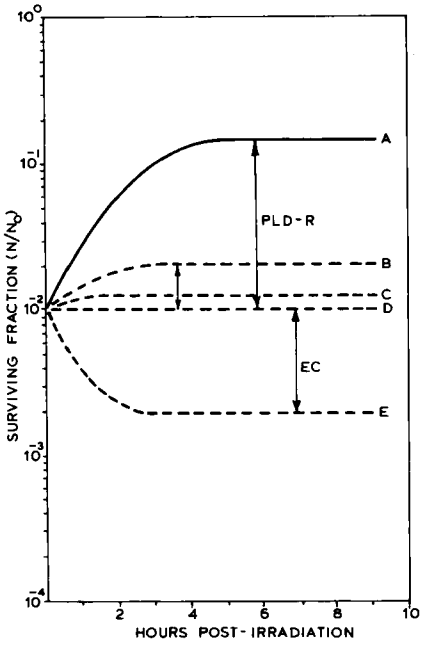

Fig. 3 The survival of irradiated mammalian cells increases if the cells are incubated after irradiation (10 Gray) prior to dilution and subculture for colony-forming-unit analysis. This is defined operationally as potentially lethal damage recovery (PLD-R). A recovery ratio of 15 is illustrated in curve A, which is similar to that reported for human cells from radioresistant tumours irradiated in culture (Ref. 58). Curve B is a recovery ratio of approximately 2, which is similar to that reported for Chinese hamster cells (Ref. 8). *cis*-DDP has been reported to produce an inhibition of PLD-R as represented by curve C (Ref. 8). Total inhibition of recovery would be expected to produce curve D. Certain platinum complexes reduce the survival to less than that expected by total inhibition of PLD-R, an effect called enhanced chemotoxicity (EC). Curve E is an example of EC reported for *cis*-DEP in Chinese hamster cells which normally would recover as in curve B without drug present (Ref. 40)

this work suggests that *cis*-DDP operates in part through reactive free radicals, including the hydrated electron and hydroxyl radical (both species of water radiolysis), in part through the interaction of radiation-induced reactive Pt(I) intermediates, and in part through alteration of Pt(II)-DNA binding during irradiation (5). The results of these studies suggest that *cis*-DDP appears to be more effective as a radiation sensitiser in free solution rather than when bound to cellular components, and irradiated solutions of *cis*-DDP (5) and certain other platinum complexes (6) are more toxic than unirradiated solutions, an effect hypothesised to be a result of free radical formation and subsequent reaction of Pt(I) intermediates.

Interactions between Platinum and Radiation in Mammalian Cells

In studies using established cell lines growing in petri dishes, *cis*-DDP produced relatively small enhancement ratios (ER) of about 1.3 in Chinese hamster V79 lung cells using 10 micromolar drug concentrations (4, 7) and in rat hepatoma cells using only 2.5 micromolar *cis*-DDP (8). Although it is anticipated that the greater than additive interaction between the *cis*-DDP and radiation may become larger at higher platinum concentrations, it is difficult to do these experiments at higher drug concentrations since the cytotoxicity from the platinum alone becomes severe. When *cis*-DDP was combined with misonidazole prior to irradiation, ER values obtained were higher than expected from the additive effects of the two radiosensitisers (7).

The addition of *cis*-DDP to cells after irradiation, at a time when free radical mediated radiosensitisation would not be expected to occur, produces an enhanced cell kill relative to the additive effects expected from the two agents (8–10). This potentiation of cell kill may result from molecular mechanisms such as the inhibition of repair of radiation damage by the platinum complexes. A classic response to radiation is the cellular recovery (increased cell survival) when plateau phase cells are irradiated and then incubated for several hours prior to

trypsinisation and subculture for viability assay. This effect is illustrated in Figure 3. This increased survival is called recovery from radiation-induced potentially lethal damage (PLD) (11). If platinum complexes inhibit the recovery from radiation damage, this may represent a second mechanism of interaction of clinical relevance since PLD repair inhibitors are currently being considered for clinical trials (12).

Therapeutic Potentiation in Animal Tumours

When *cis*-DDP is injected into mice bearing transplantable mouse mammary tumours (MTG-B) or rats bearing intracerebrally implanted rat brain tumours (RBT) prior to radiation therapy, an enhanced antitumour effect is produced (13). In these experimental systems it is difficult to prove that the result is supra-additive, that is greater than the sum of the effects produced by either agent acting alone. However, evidence for potentiation of the radiation therapy by *cis*-DDP in animal tumour systems has been confirmed in several laboratories (14–23) and the enhanced tumouricidal effects have represented a therapeutic gain since they have not been accompanied by an equivalent enhancement of normal tissue damage. An ER value of 1.7 was reported for single combinations of *cis*-DDP and radiation therapy using tumour cure as an assay endpoint in a mouse tumour system (14). Since conventional clinical radiotherapy is administered in multiple fractions of irradiation, mouse tumours were treated with multiple fractions of *cis*-DDP in conjunction with multiple radiation fractions, producing an ER of 1.9 (19). In this study using fractionated doses *cis*-DDP was one of only two drugs to produce supra-additivity with the X-ray enhancement dependent upon the timing of the drug to the radiation therapy.

In most studies which combined *cis*-DDP with radiation, skin did not show significant potentiation of damage (14, 16, 19). A small enhancement of skin damage was reported in two studies (24, 25). Duodenal crypt cells in mouse intestine showed only a moderate enhancement of radiation-induced damage when the platinum was added to the irradiation (16, 17, 19, 26), including inhibition of radiation-induced repair by *cis*-DDP (27–28). The lack of evidence for significant enhancement in these two important normal tissues is encouraging, since significant levels of platinum have been reported in mouse skin (29).

The Potential Represented by Second Generation Platinum Complexes

The therapeutic potentiation reported in preclinical studies may represent a lower limit of the level of enhancement which is potentially attainable by using less toxic second generation platinum chemotherapy, such as Carboplatin or Iproplatin, or by using novel metal complexes designed as radiosensitisers. Radiosensitisation of hypoxic cells has been reported for several platinum complexes (for reviews see references 30–32), including Carboplatin (6), as well as the *trans*-DDP isomer (4, 6) which lacks significant antitumour activity. Although it is not known what the active chemical species is or whether binding to DNA is important in radiosensitisation, combining less toxic platinum complexes with radiation has resulted in efficient ER values (4, 6).

In animal test systems higher doses of some of these platinum complexes may be administered and the levels are not necessarily limited by kidney toxicity. Using these complexes it should be possible to produce higher peak plasma levels and correspondingly higher platinum levels in solid tumours. This is demonstrable in mice where administration of a five-fold dose of Carboplatin relative to *cis*-DDP results in approximately a similar ratio in total platinum levels in human tumours growing as xenografts in these animals (33). In the MTG-B mouse tumour model the less toxic *trans*-DDP was shown to be a potentiator of radiation-induced tumour cell kill and this effect was greater when the population of hypoxic cells was increased in the tumour during irradiation (34).

The interactions between Iproplatin and

radiation have been studied extensively in the laboratories of Nias and his co-workers (for review see reference 35). These investigators selected this platinum complex because of its favourable therapeutic index in early pre-clinical studies, and they realised that this complex would become a likely candidate for clinical trials. ER values as high as 1.9 were reported when hypoxic Chinese hamster ovary (CHO) cells were irradiated following certain time periods and specific concentrations of the drug. However, under other conditions potentiation of radiation-induced cell kill was reported for well-oxygenated CHO cells (36). The results of these studies emphasise that platinum co-ordination complexes have a complicated pattern of interaction with radiation that is dependent upon variables such as drug concentration, time intervals between drug and radiation, oxygenation status of the cell, and cell culture conditions.

Probably the largest ER values (2.4) reported to date were obtained in cultured hypoxic cells using FLAP, a platinum complex which attaches two of the electron affinic 5-nitroimidazole metronidazole moieties to dichloroplatinum(II) (37). Other investigators failed to reproduce this effect but reported that if intracellular glutathione levels are depleted using diethylmaleate (DEM), the ER produced by FLAP can be increased further (38). Glutathione with its sulphydryl groups would be expected to react with free radicals as well as provide a binding site for platinum complexes. A similar approach to designing a radiosensitiser was the synthesis by Teicher of *trans*-dichlorobis(azomycin)platinum(II), or 2NIPt. This is a relatively non-toxic complex which incorporates two of the electron affinic azomycin moieties, and which proved to be an effective hypoxic cell radiosensitiser in bacteria (6) and in mammalian cells (39). In the bacterial study a 200 micromolar concentration of 2NIPt was 2.5 times more effective in sensitising hypoxic cells than 400 micromolar of azomycin alone. An agent that binds to DNA such as a platinum complex might serve as a carrier of an active radiosensitiser to the DNA. However, the relative roles of the free solution platinum complex relative to DNA-bound platinum have not been identified for mammalian cells.

Certain platinum complexes may prove to be very effective potentiators when administered after irradiation. An example is *cis*-dichlorobis-(ethylenimine) platinum(II), or *cis*-DEP, which produces an enhanced cell kill greater than expected on the basis of total inhibition of PLD recovery (40). This effect has been called "enhanced chemotoxicity", or EC, since V79 cells which have been irradiated appear to be more sensitive to subsequent exposure to the platinum complex. Since a platinum complex administered to cells or to tumour bearing animals before irradiation would also be present in the interval immediately following irradiation, the EC effect may be partially responsible for some of the platinum-radiation interactions reported for cells and animal tumours, as well as being a contributing factor in the responses of human tumours that have been reported in preliminary clinical trials.

Results of Clinical Trials

The administration of *cis*-DDP is currently being co-ordinated with radiation therapy in a number of clinical studies including the treatment of brain tumours (41, 42), head and neck tumours (43–48), malignant melanomas (48) and bladder cancers (49), in order to exploit the interaction of platinum with radiation. These studies are summarised in the Table. The results of these trials are preliminary and most of the studies are testing toxicity of the combined treatment rather than evaluating the efficacy of the new protocol compared to the effects of either agent alone. However, the results show promise in that this combination may be resulting in some cases in improved responses of the patients' tumours to therapy, including the eradication of bulky disease or the reduction of bulky tumours to a level potentially manageable by surgery or higher doses of radiation (44). It is important to note that these clinical studies have been designed without knowing the precise mechanisms for the interactions or the optimum timing and

dose relationships between the two modalities.

Pharmacokinetic studies of platinum levels after i.v. infusion of *cis*-DDP report that peak plasma levels have short halftimes of approximately 0.5 hour (50). The authors of one of the clinical studies (44) have suggested that favourable responses in preliminary trials to date probably reflect radiation potentiation mechanisms other than radiosensitisation of hypoxic cells. Concentrations of platinum have been measured in some human tissues and tumours. Levels such as 6.4 micrograms platinum per gram of wet tissue for a squamous cell carcinoma (51) begin to approach the levels of *cis*-DDP required to produce a small interaction (ER) in cultured cells. Furthermore, a peak of platinum concentration was measured in tumours of patients six hours after injection of the *cis*-DDP which was greater than tumour platinum levels at one hour and 24 hours. This concentration is greater than could be explained on the basis of intravascular plasma levels. If this represents an accumulation by the tumour there may be a time period most appropriate for delivering the radiation therapy when the appropriate platinum species is most concentrated or when tumour platinum levels may be higher than normal tissue levels. Single clinical doses in excess of 400 mg/m^2 of Carboplatin are tolerated (52, 53) compared to doses of about 100 mg/m^2 of *cis*-DDP. Peak plasma levels of 31 micromolar total platinum have been reported in patients after a dose of only 150 mg/m^2 of Carboplatin with ultrafilterable (free) platinum concentrations of 15 micromolar (54). Not only are the levels of platinum higher, but Carboplatin differs from *cis*-DDP in that the free platinum species is present for longer periods of time in the plasma. This may represent a clinically important platinum species for interacting with radiation, as has previously been suggested (6, 44).

Conclusion

Observations of enhancements of radiation effects by *cis*-DDP in animals reported in the early 1970s by Zak and Drobnik (55) and Wodinsky and his co-workers (56) signalled the potential for interaction between these two agents. These interactions have been studied in a variety of systems and a rationale has been developed for combining platinum chemotherapy with radiation therapy to exploit radiosensitisation and potentiation. Promising new second generation platinum complexes may produce similar interactions. It is anticipated that the lower toxicity of these drugs and the persistence of free platinum complexes will permit higher levels of platinum to reach tumours in a state appropriate for greater interaction (6). In addition, new techniques are being developed which may permit more platinum to reach tumour cells, including the use of chloruresis (57), rescue of normal tissues from platinum-induced toxicity (20), and the design of non-toxic and/or targeted platinum complexes with radiosensitiser (6, 37, 39) or repair inhibitor moieties.

Assuming that tumours are treated with radiation to the tolerance of normal tissues in the irradiation treatment field, the addition of platinum complexes must increase the therapeutic ratio, that is increase the effect on the tumour without a corresponding enhancement of normal tissue damage. In summation, three possible provisions would be (a) preferential radiosensitisation of hypoxic tumour cells if normal tissues are well oxygenated; (b) inhibition of repair of radiation damage or enhanced chemotoxicity if the effect is preferential to tumours; and (c) radiosensitisation or potentiation with preferential concentration or activity of the appropriate drug species in the tumour relative to normal tissues at the appropriate time for irradiation.

The role of PLD recovery in clinical radiocurability is still uncertain but the potential use of PLD repair inhibitors is being considered since the radioresistance (poor radiocurability) of certain human tumours has been correlated, at least in petri dishes, with a high proficiency for PLD recovery (58). Cultured melanoma cells, for example, demonstrate significant PLD recovery after radiation doses used clinically (59, 60). Furthermore, radioresistant tumour cells distant from blood

Preliminary Clinical Studies Using *cis*-DPP with Radiation			
Authors	Tumours treated	Patients reported	Reference
Creagen *et al.*	Advanced head and neck	3	47
Reimer *et al.*	Melanoma	7	48
	Oat cell lung	1	
	Squamous cell head and neck	2	
	Bladder	1	
	Pleural mesothelioma	1	
	Metastatic carcinoma	1	
Stewart *et al.*	Brain	15	41
Leipzig	Head and neck	14	46
Pinedo *et al.*	Head and neck	6	45
Herr *et al.*	Bladder	24	49
Golding and van Zanten	Squamous cell lung	1	*
Keizer *et al.*	Advanced squamous cell	18	**
	Advanced adenocarcinoma	9	
Showel *et al.*	Advanced head and neck	20	**
Deneufbourg	Advanced head and neck	55	**
Moylan *et al.*	Non-oat cell carcinoma	10	***
Coughlin *et al.*	Head and neck	25	43
Coughlin and Richmond	Head and neck	21	44

* *Br. J. Radiol.*, 1983, **56**, 281–282
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*** Abstract in *op. cit.*, Ref. 39, 350

vessels are likely to be in a deficient nutrient and metabolic condition which may favour PLD recovery (11,61). At this time it is not known if platinum complexes will reach these cells in human tumours.

It should be cautioned that the variety of responses reported between laboratories, platinum complexes, and cell lines may also suggest that interactions at the clinical level may be both complicated and elusive. The mechanisms for the platinum-radiation interactions need to be elucidated if enhancement of normal tissue damage is to be avoided and if the potential for this combination is to be realised. Other metals may even be superior to platinum for these effects. It is apparent that the field of inorganic chemistry has taken an additional important role in cancer therapy.

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