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Interaction of D, L- and D-Tetraplatin with Hyperthermia *in vitro* and *in vivo*

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The cytotoxicities of D,L-tetraplatin and D-tetraplatin were evaluated at 37°C, 42°C and 43°C at normal pH, at pH 6.45 and under normally oxygenated and hypoxic conditions in EMT-6 cells *in vitro*. The D-isomer was also tested in FSaIIC cells *in vitro*. Under these various conditions the pure D-isomer was very similar in cytotoxicity with the racemic mixture. Like cisplatin, both D,L- and D-tetraplatin were selectively cytotoxic toward normally oxygenated cells under acidic pH (6.45) conditions at 37°C. In both cell lines the cytotoxicity of D,L- and D-tetraplatin was markedly increased at hyperthermic temperatures. Under the same conditions platinum levels in EMT-6 cells exposed to D,L- or D-tetraplatin were higher than in cells exposed to cisplatin, and unlike cisplatin there was an increase in intracellular platinum levels when the cells were exposed to D,L- or D-tetraplatin at 42°C compared with 37°C. The tumour growth delay of the FSaIIC fibrosarcoma was the same for D,L- and D-tetraplatin. A dose of 10 mg/kg intraperitoneally of tetraplatin produced a tumour growth delay of about 4.3 days which was increased to about 6 days with the addition of local hyperthermia (43°C, 30 min) to the drug treatment. The tumour cell survival assay also showed no difference between D,L- and D-tetraplatin and a log-linear increase in tumour cell killing with increasing drug dose which was increased 1.5-3-fold with local hyperthermia. D,L- and D-tetraplatin were relatively much more cytotoxic toward bone marrow colony forming units of granulocyte–macrophage progenitors (CFU-GM) than was cisplatin and this cytotoxicity was increased about 5–10-fold under hyperthermic conditions. There was an increase in DNA crosslink formation in tumours when hyperthermia accompanied tetraplatin treatment. Overall, D,L- and D-tetraplatin produced very similar responses under hyperthermic conditions in both tumour and normal tissues, and may be a useful agent in combination with local hyperthermia.
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INTRODUCTION

MANY ANTICANCER drugs used clinically demonstrate significantly increased cell killing at elevated temperatures. Four excellent reviews of this area have been published [1–4]. In general, anticancer drugs whose cytotoxicities are increased at elevated temperatures show greater supra-additive lethality as the temperature of exposure increases [5–10]. The mechanisms responsible for these temperature effects on cell killing by anticancer drugs are not entirely understood. It is not known whether the net increase in DNA damage thought to underlie the interaction between hyperthermia and most anticancer drugs is due to an increase in drug uptake, alterations in intracellular distribution or metabolism, an increase in drug reaction rates with DNA, or heat-induced inhibition of DNA repair [10, 11]. It is possible (and even probable) that more than one of these mechanisms may be operating for any particular drug at elevated temperatures.

The greatest enhancement by hyperthermia (43°C/30 min locally) in FSaIIC fibrosarcoma tumour cell killing *in vivo* which we have observed with any of the antitumour agents occurred with cisplatin [12–16]. There was more than 2 logs increased tumour cell killing with cisplatin and 43°C, 30 min, than with cisplatin alone at normal therapeutic doses of the drug [13]. In cell culture, the enhancement in cisplatin cytotoxicity with heat varies markedly with the environmental conditions (oxygenation, pH) during drug exposure [12]. Carboplatin has also shown significant enhancement in cell killing with hyperthermia [17, 18].

Tetraplatin is a second-generation platinum complex in which platinum is in the +4 oxidation state and which exists in 2 isomeric forms, D- and L- [19]. Most preclinical studies, both *in vitro* [19–23] and *in vivo* [24, 25] have been conducted with the racemic mixture (1:1, D:L) of the two tetraplatin isomers. In several *in vitro* studies, tetraplatin has been a more potent cytotoxin than cisplatin and the degree of crossresistance of cisplatin-resistant cell lines to tetraplatin has been less than to other platinum-containing anticancer agents, especially carboplatin [21–23]. We also found that D-tetraplatin was, in general, a more potent cytotoxic agent in cell culture than cisplatin or carboplatin, as determined by comparison of the drug concentrations required to kill 1 log of cells [26]. D-tetraplatin has previously been shown to be the more potent cytotoxin of the two tetraplatin isomers in a human myeloma cell line [22] and two human ovarian cell lines [21]. Both tetraplatin isomers are rapidly biotransformed [27–29] and in rats the racemic mixture has been shown to be less nephrotoxic than cisplatin [30, 31]. Thus tetraplatin may have substantial clinical potential.

In this report we describe the effect of hyperthermia on the cytotoxicity of D,L- and D-tetraplatin under various environ-

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mental conditions *in vivo* and on the effect on the tumour growth delay and tumour cell and bone marrow survivals of the FSaIIC fibrosarcoma *in vivo*.

MATERIALS AND METHODS

Drugs

Cisplatin was a gift from Drs Donald H. Picker and Michael J. Abrams, Johnson Matthey (West Chester, Philadelphia). D, L- tetraplatin and D- and L-tetraplatin were gifts from Dr J. Patrick McGovren, Upjohn (Kalamazoo, Michigan).

Cell culture

EMT6 murine mammary tumour cells have been widely used for the study of hypoxia [32, 33] and heat effects *in vitro* [34]. Cultures were maintained in exponential growth in Waymouth's medium (ISI, Chicago), supplemented with 15% newborn calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) (Grand Island Biological, Grand Island, New York). FSaIIC murine fibrosarcoma cells were maintained in exponential growth in Alpha medium (Grand Island Biological), supplemented with 10% fetal bovine serum (Sigma), penicillin and streptomycin as above [35, 36]. *In vitro* plating efficiencies of control cultures were 65–80%.

Heat treatments

Exponentially growing cells were exposed to a temperature of 37°, 42°, or 43° for 1 h in a Plexiglass water tank with a continuous in-flow and out-flow system controlled by a water temperature controller (Braun Thermomix 1460; Braun Instruments) [37]. Cells underwent heating in sealed plastic flasks (Falcon) containing 5 ml complete medium. Water temperature could be maintained within 0.10°C.

Production of hypoxia

To produce hypoxia, the plastic flasks, containing exponentially growing monolayers in complete medium plus serum, were fitted with sterile rubber septums and exposed to a continuously flowing 95% N_2/5% CO_2 humidified atmosphere for 4 h at 37°C as previously reported [38, 39]. Parallel flasks were maintained in 95% air/5% CO_2. At the end of 4 h, the drug or vehicle was added to the flasks by injection through the rubber septum without disturbing the hypoxia.

pH alterations

The pH of the medium was adjusted using a sodium bicarbonate ($NaHCO_3$)/5% CO_2 buffer system [40].

Drug treatments

Exponentially growing cells were exposed to varying concentrations of D,L-tetraplatin or D-tetraplatin in T-25 flasks for 1 h at 37°, 42° or 43°C. Non-drug-treated controls were handled identically. Drugs were prepared in sterile PBS immediately before use and added to the flasks in a small volume (50–100 μl).

Cell viability measurements

Cell viability was measured by the ability of single cells to form colonies *in vitro*, as described previously [38, 39]. Each experiment was repeated three times and each data point per experiment represents the results of three different dilutions of cells plated in duplicate.

Platinum determinations

Solutions of cisplatin, D,L-tetraplatin and D-tetraplatin were prepared in medium without serum. The final concentration of all three solutions was confirmed by flameless atomic absorption spectrophotometry [41]. EMT6 cells in exponential growth were trypsinised with a 0.25% trypsin solution and centrifuged at 500 *g* for 4 min. The cell pellets were resuspended in medium containing 15% newborn calf serum at a concentration of approximately 4×10^6 cells/ml for 1 h. Tubes containing the drugs in medium were preheated in water baths at 37°C and 42°C. The cells were added to the drug-containing medium at each temperature and were incubated for 1 h at a concentration of 1.1×10^6 cells/ml of 25 μmol/l drug solution. After incubation, the cells were placed on ice and washed 4 times with PBS to remove extracellular drug. The final washings were determined by atomic absorption spectrophotometry to have below detectable levels of platinum. The final cell pellet was sonicated, and the mass of intracellular platinum was determined by atomic absorption spectrophotometry.

Flameless atomic absorption spectrophotometry procedure [13]

Platinum from a 15-μl sample injection volume was atomised from the walls of pyrolytically coated graphite tubes. A Perkin Elmer Model 2380 atomic absorption spectrophotometer was used in conjunction with a Perkin Elmer Model 400 graphite furnace to measure the absolute mass of platinum in the cell samples [41, 42]. Each measurement was made in triplicate in three independent experiments.

Tumour

The FSaII fibrosarcoma [35] adapted for growth in culture (FSaIIC) [36] was carried in male C3H/FeJ mice (the Jackson Laboratory, Bar Harbor, Maine). For the experiments, 2×10^6 tumour cells prepared from a brei of several stock tumours were implanted intramuscularly into the legs of male C3H/FeJ mice, 8–10 weeks of age. This tumour is non-antigenic in the C3H/FeJ host.

Tumour growth delay experiments

When the tumours were approximately 100 mm^3 in volume (6–7 mm diameter; [32]), treatment was initiated. In those groups receiving the drug, D,L-tetraplatin (3, 5, 7, 10 or 15 mg/kg) or D-tetraplatin (3, 5, 7, 10 or 15 mg/kg) in PBS (0.2 ml) was injected as a single dose intraperitoneally. The maximum tolerated dose of D,L- or D-tetraplatin was 15 mg/kg. In those groups receiving hyperthermia, heat was delivered as a single dose locally to the tumour-bearing limb by emersion in a specially designed Plexiglass waterbath at 44°C which allowed the centres of tumour to reach 43 (S.D. 0.2)°C as measured by a digital readout thermistor (Sensortech, Clifton, New Jersey) placed into the centre of the tumour in selected control animals as previously described [13]. No anaesthetic was used. Hyperthermia was delivered immediately following intraperitoneal injections. The progress of each tumour was measured thrice weekly until it reached a volume of 500 mm^3. Untreated control tumours reached 500 mm^3 12–14 days post implantation. Tumour growth delay was calculated as the days taken by each individual tumour to reach 500 mm^3 compared with the untreated controls. Each treatment group had seven animals, and the experiment was repeated three times. Days of tumour growth delay are the mean (S.D.) for the treatment group compared with the control.

Tumour excision assay

When the tumours were approximately 100 mm^3 in volume (about 1 week after tumour cell implantation), the animals were

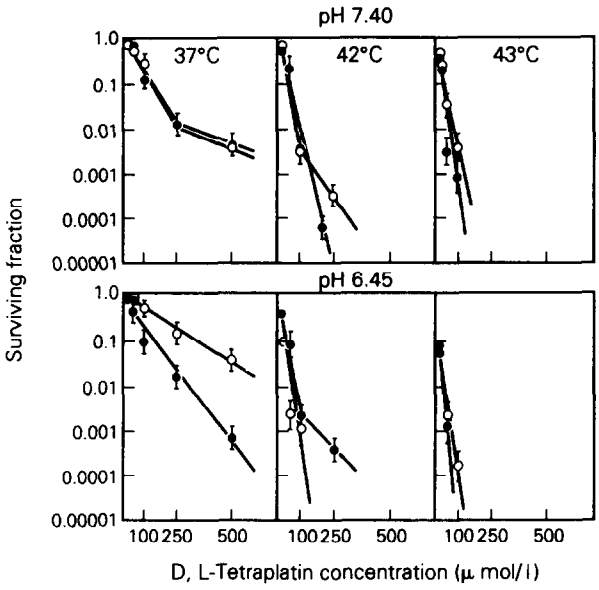

Fig. 1. Survival curve of exponentially growing normally oxygenated (●) and hypoxic (○) EMT-6 cells exposed to D, L-tetraplatin at 37°C, 42°C, or 43°C at pH 7.40 and pH 6.45. The survival value plotted on the *y*-axis represents heat-alone killing at the conditions indicated. Mean (S.D.) of three independent experiments.

given injections intraperitoneally of various doses of D,L-tetraplatin (10, 20, 30 or 40 mg/kg) or D-tetraplatin (10, 20, 30 or 40 mg/kg) alone or immediately followed by hyperthermia (43°C, 30 min), as described above, to the tumour-bearing limb. Mice were killed 24 h after treatment to allow for full expression of drug cytotoxicity and repair of potentially lethal damage. The tumours were excised, and single cell suspensions were prepared as described previously [14, 43]. The untreated tumour cell suspensions had a plating efficiency of 10–16%. The results are expressed as the surviving fraction (S.E.) of cells from treated groups compared with untreated controls [13–15].

Bone marrow toxicity

Bone marrow was taken from the same animals used for the tumour excision assay. A pool of marrow from the femurs of two animals was obtained by gently flushing the marrow through a 23-gauge needle and CFU-GM assay was carried out as described previously [43, 44]. Colonies of at least 50 cells were scored on an Acculite colony counter (Fisher Scientific, Springfield, New Jersey). The results from three experiments, in which each group was measured at three cell dilutions in duplicate, were averaged. The results are expressed as the surviving fraction (S.E.) of treated groups compared with untreated controls.

Alkaline elution

FSaIIC fibrosarcoma-bearing mice as described above were given injections of 4.63 kBq/g [methyl-^{14}C]thymidine (185 MBq/mol; New England Nuclear, Boston) 24 h prior to drug treatment with CDDP (10 mg/kg), D,L-tetraplatin (10 mg/kg) or D-tetraplatin (10 mg/kg) in the presence or absence of hyperthermia (43°C, 30 min). 24 h after treatment the tumours were excised, and a single cell suspension was prepared as described previously for the tumour excision assay [43]. Alkaline elution was performed by standard procedures [45].

RESULTS

At normal pH (pH 7.40) and 37°C, like cisplatin [12], D,L-tetraplatin was equally cytotoxic toward normal oxygenated and hypoxic EMT-6 cells (Fig. 1). D,L-tetraplatin was less cytotoxic than cisplatin in these cells killing less than 1 log of cells at a concentration of 100 μmol/l whereas 100 μmol/l of cisplatin under the same conditions killed 3 logs of EMT-6 cells [12]. The cytotoxicity of D,L-tetraplatin was markedly increased under hyperthermic conditions in both normally oxygenated and hypoxic cells. At 42°C (pH 7.40), 100 μmol/l of D,L-tetraplatin killed about 2.5 logs of cells. There was little further increase in cytotoxicity at 43°C (pH 7.40).

D,L-tetraplatin was more cytotoxic toward normally oxygenated cells that toward hypoxic cells at 37°C and pH 6.45. Increasing the temperature during drug exposure to 42°C markedly increased the cytotoxicity of the D,L-tetraplatin such that 100 μmol/l of the drug killed about 3 logs of cells compared with <0.5-1 log of cells at 37°C. The cytotoxicity of D,L-tetraplatin was further increased at 43°C (pH 6.45) and the combination of drug (100 μmol) and heat killed at least 4 logs of cells under both oxygenation conditions.

The D-isomer of tetraplatin showed a degree of cytotoxic selectivity for normally oxygenated EMT-6 cells compared with hypoxic EMT-6 cells at 37°C and pH 7.40 but was not overall more cytotoxic than the racemic mixture under these conditions (Fig. 2). Like the isomeric mixture, the cytotoxicity of D-tetraplatin was markedly increased at 42°C with a smaller further increase in cytotoxicity at 43°C. A concentration of 100 μmol/l of D-tetraplatin killed about 2.5 logs of cells at 42°C (pH 7.40). At each temperature D-tetraplatin was slightly less cytotoxic toward hypoxic cells.

Under acidic pH (pH 6.45) conditions and 37°C D-tetraplatin was markedly selectively cytotoxic toward normally oxygenated cells. At a concentration of 100 μmol/l D-tetraplatin the differential in the killing of normally oxygenated cells compared to hypoxic cells was 1 log (1.5 logs vs. 0.5 log). When drug exposure (100 μmol/l) was carried out at 42°C (pH 6.45) there was a 3 log increase in the killing of hypoxic cells and about a 1.5 log increase in the killing of normally oxgenated cells. At 43°C

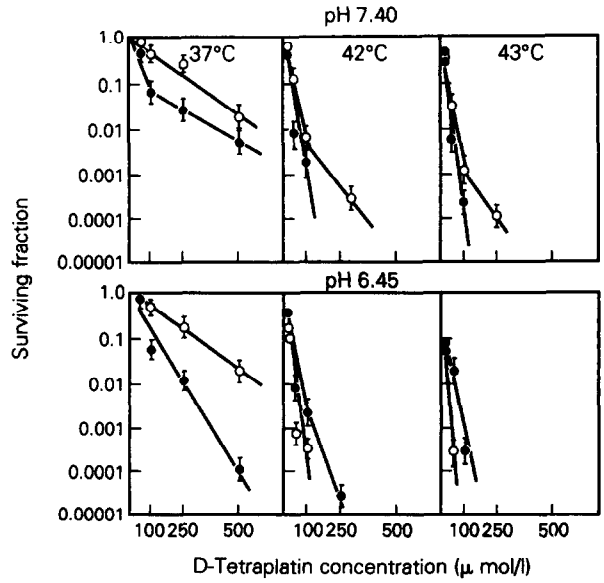

Fig. 2. Survival curve of exponentially growing normally oxygenated (●) and hypoxic (○) EMT-6 cells exposed to D-tetraplatin at 37°C, 42°C or 43°C at pH 7.40 and pH 6.45.

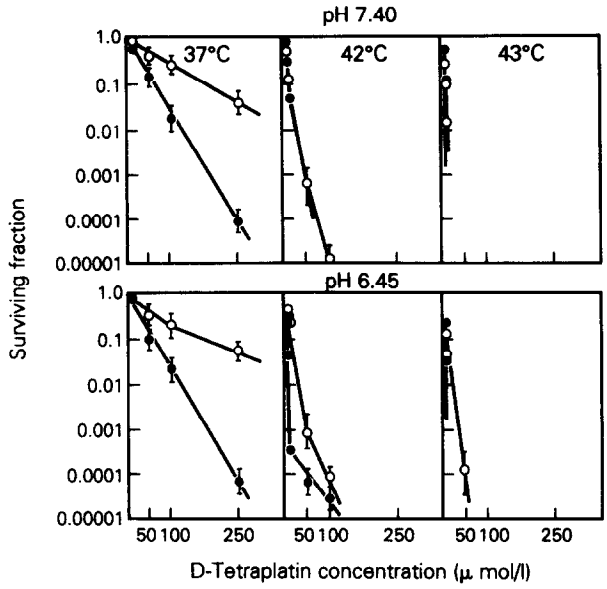

Fig. 3. Survival curve of exponentially growing normally oxygenated (●) and hypoxic (○) FSaIIC cells exposed to D-tetraplatin at 37°C, 42°C or 43°C at pH 7.40 and pH 6.45.

(pH 6.45) the combination of D-tetraplatin and heat killed almost 4 logs of normally oxygenated cells and more than 5 logs of hypoxic cells.

By comparison, FSaIIC cells were somewhat more sensitive to D-tetraplatin than were EMT-6 cells under the same conditions (Fig. 3). Under normal pH (pH 7.40) conditions and 37°C nearly 2 logs of normally oxygenated FSaIIC cells and more than 0.5 log of hypoxic FSaIIC cells were killed by 100 μmol/l D-tetraplatin. The increase in the cytotoxicity of D-tetraplatin was even further increased by hyperthermic conditions during drug exposure in the FSaIIC cells than in the EMT-6 despite the fact that FSaIIC cells and EMT-6 cells were approximately equally affected by the hyperthermia treatments alone. When FSaIIC cells were exposed to D-tetraplatin (100 μmol/l) at 42°C (pH 7.40) 5 logs of hypoxic cells were killed and more than 5 logs of normally oxygenated cells were killed. If the drug exposure was carried out at 43°C (pH 7.40) 2 logs of both normally oxygenated or hypoxic cells were killed under 10 μmol/l of D-tetraplatin.

Under acidic pH (pH 6.45) and 37°C conditions D-tetraplatin was equally as effective against FSaIIC as at normal pH (pH 7.40) and, again, was selectively cytotoxic toward the normally oxygenated cells. Increasing the temperature during drug (100 μmol/l) exposure to 42°C and (pH 6.45) resulted in 4.0–4.5 logs of cell killing. When the temperature during drug exposure was further increased to 43°C (pH 6.45), 10 μmol/l D-tetraplatin killed about 1.5 logs of both normally oxygenated and hypoxic cells.

Under the same exposure conditions, normally oxygenated EMT-6 contained higher levels of platinum after exposure to D,L-, D- or L-tetraplatin than after exposure to cisplatin (Table 1). There was no significant difference among the levels of platinum found in the EMT-6 cell depending on the isomer or isomeric mixture of tetraplatin used. Interestingly, although there was no significant increase in the level of platinum in the EMT-6 exposed to cisplatin at 42°C (pH 7.40 or pH 6.45) compared with 37°C, there was a 1.6–1.8-fold increase in the amount of platinum found in the cells after exposure to the various forms of tetraplatin at 42°C compared with 37°C. The pH of the extracellular environment was not a significant factor influencing the intracellular levels of platinum for any of the complexes tested.

Tumour growth delay studies were carried out with various single doses of D,L- or D-tetraplatin in the FSaIIC fibrosarcoma with or without local hyperthermia (43°C, 30 min) to the tumour-bearing limb (Fig. 4). There was no difference in the tumour growth delay produced by D,L- or D-tetraplatin. The tumour growth delay increased over the dosage range examined from about 0.5 day with 3 mg/kg to about 5 days with 15 mg/kg. The addition of hyperthermia to treatment with these drugs resulted in an increase in tumour growth delay from about 2.5 days to 4.5 days with 5 mg/kg of tetraplatin and from about 4.3 days to about 6 days with 10 mg/kg of tetraplatin. The tumour growth delay produced by the hyperthermia treatment alone was about 1.4 days [14]. The dose of 10 mg/kg was the maximum tolerated dose of tetraplatin with local hyperthermia.

Tumour cell and bone marrow CFU-GM survivals were assayed from animals bearing the FSaIIC fibrosarcoma after treatment with various doses of D,L- or D-tetraplatin with or without local hyperthermia (43°C, 30 min) (Figs 5 and 6). As with the tumour growth delay studies, there was no difference in the tumour cell killing obtained after treatment of the tumour-bearing animals with D,L- or D-tetraplatin. The tumour cell killing with both drugs increased in a log-linear manner over the dosage range examined. The addition of hyperthermia to treatment with D,L- or D-tetraplatin increased tumour cell killing by these drugs by 1.5–3-fold over the drug dosage range tested.

Both D,L- and D-tetraplatin were more cytotoxic toward bone marrow CFU-GM than toward FSaIIC tumour cell *in vivo* over the dosage range from 10–30 mg/kg. The addition of hyperthermia to treatment with either D,L- or D-tetraplatin increased the killing of bone marrow CFU-GM from the femurs of the tumour-bearing limb by 5.4–10.8-fold over the dosage range of the drug examined.

DNA alkaline elution was used to assess DNA crosslinking in FSaIIC tumours treated with 10 mg/kg of cisplatin, D, L-tetraplatin or D-tetraplatin. Using this methodology, the crosslinking factor obtained for cisplatin treatment was 1.7 and for D,L- and D-tetraplatin were 2.9 and 4.0, respectively. The amount of tumour cell killing obtained with these various treatments, however, was quite comparable indicating that lesions formed by cisplatin may be more lethal than those formed by the tetraplatins. There was between a 2.2 and 2.7-fold increase in DNA crosslinking factor when hyperthermia treatment followed administration of each of the platinum complexes. However, with cisplatin this increase in DNA cross-linking accompanied about a 2 log increase in tumour cell killing, whereas with the tetraplatins only about a 1.5-fold increase in tumour cell killing resulted.

DISCUSSION

Various physiological environments exist in solid tumour masses which may affect the metabolic status of tumour cells and the actions of antitumour agents [46–48]. We are continuing to search for drugs capable of becoming very cytotoxic in conjunction with hyperthermia across various environmental conditions. Although D,L- and D-tetraplatin have been shown to be more potent cytotoxic agents than cisplatin in several human tumour cell lines [26], in both EMT-6 cells and FSaIIC cells D,L- and D-tetraplatin were less potent cytotoxic agents than cisplatin. The degree of hyperthermic sensitisation of

Table 1. Comparison of IC_{90}s and intracellular levels of platinum after exposure of EMT-6 cells to various platinum complexes at 37°C or 42°C for 1 h under normally oxygenated conditions

	IC90(μmol/l)*				Platinum levels (ng/10^6 cells)†			
	pH 7.40		pH 6.45		pH 7.40		pH 6.45	
Treatment	37°C	42°C	37°C	42°C	37°C	42°C	37°C	42°C
Cisplatin	22	4	40	5	1.0(0.2)‡	1.2(0.2)	1.1(0.1)	1.2(0.1)
D, L-tetraplatin	140	50	150	40	3.9(0.4)	6.8(0.5)	4.1(0.4)	7.3(0.5)
D-tetraplatin	90	35	125	25	4.2(0.4)	6.7(0.5)	4.6(0.4)	7.3(0.5)
L-tetraplatin	170	65	200	75	3.7(0.4)	5.8(0.4)	3.5(0.3)	5.8(0.5)

*Micromolar concentration of drug required to reduce cell survival by 90% after 1 h exposure to the drug.
†EMT-6 cells were exposed to 25 μmol of drug for 1 h. Platinum levels were measured by flameless atomic absorption spectrophotometry. By analysis of variance, no statistically significant temperature-dependent effect on drug accumulation was observed at the $P < 0.01$ level for D,L-tetraplatin, D-tetraplatin and L-tetraplatin but not cisplatin.
‡Mean (S.E.).

normally oxygenated cells at normal pH was greater for cisplatin than for tetraplatin but the degree of hyperthermic sensitisation of hypoxic cells at normal pH was greater for tetraplatin than for cisplatin. Both D,L- and D-tetraplatin, like cisplatin, were more cytotoxic toward normally oxygenated cells in an acidic environment than toward hypoxic cells. This finding provides some indication that these drugs may spare the hypoxic cells in solid tumour masses which are likely to be in an acidic environment [47–49]. Using the JM human acute lymphoblastic leukaemia cell line, Cohen and Robins [50] found essentially maximal thermal enhancement of tetraplatin cytotoxicity at 42°C with no significant increase at 43°C. The pattern of increased cell killing with hyperthermia in EMT-6 cells and in FSaIIC cells was similar to that seen in the JM cells with a large increase in cytotoxicity at 42°C compared with 37°C and little further change at 43°C.

Tetraplatin has previously been shown to enter *E. coli* more readily than cisplatin [51]. We found higher intracellular levels of platinum in EMT-6 cells exposed to tetraplatin than in cells exposed to cisplatin at the same concentration for the same time period. There was no increase in the uptake of cisplatin at 42°C compared with 37°C, however, there was a significant increase in intracellular platinum in the cells exposed to tetraplatin at 42°C, the major effect of heat is probably due to mechanisms which relate to increased reactivity with DNA [41] and/or to inhibition of DNA repair [11].

In vivo in the FSaIIC fibrosarcoma there was no difference in the tumour growth delay produced by D,L- or D-tetraplatin in single doses either alone or in combination with hyperthermia treatment. The growth delay produced in this tumour with 5 mg/kg of cisplatin was 4.4 (S.D. 0.9) day which was increased to 5.9 (1.1) days with the addition of hyperthermia (43°C, 30 min) to the drug treatment [13]. The tumour growth delay produced by 10 mg/kg of tetraplatin in the FSaIIC fibrosarcoma was 4.3 (0.8) days which was increased to 6.0 (0.8) days with the addition of hyperthermia (43°C, 30 min) to the drug treatment. Therefore, although tetraplatin is less potent *in vivo*, in the tumour growth delay experiment tetraplatin and cisplatin

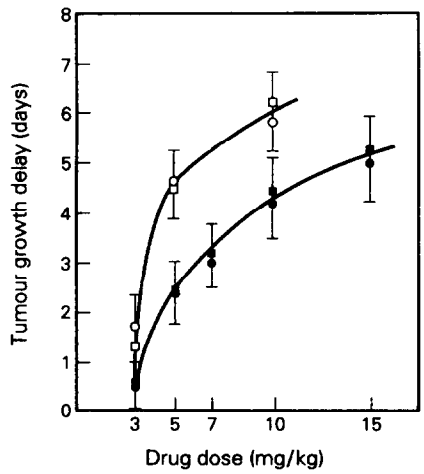

Fig. 4. Growth delay of FSaIIC fibrosarcoma produced by various doses of D,L-tetraplatin (●, ○) or D-tetraplatin (■, □) with local hyperthermia (43°C, 30 min) (○, □) or without hyperthermia (●, ■). The tumour growth delay produced by the hyperthermia treatment alone was about 1.4 days [14]. Mean (S.E.) of 14 animals.

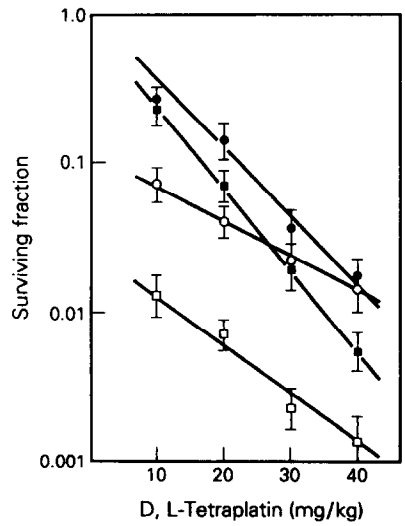

Fig. 5. Survival of FSaIIC cells from FSaIIC tumours (●, ■) and bone marrow CFU-GM (○, □) treated with various doses of D, L-tetraplatin with (■, □) hyperthermia (43°C, 30 min) delivered immediately after drug administration or without (●, ○) hyperthermia. Mean (S.E.) of three independent experiments.

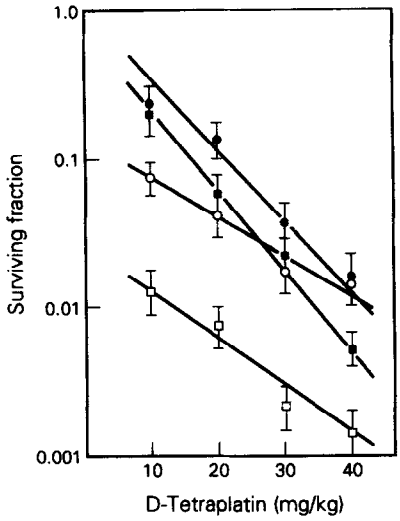

Fig. 6. Survival of FSaIIC cells from FSaIIC tumours (●, ■) and bone marrow CFU-GM (○, □) treated with various doses of D-tetraplatin with (■, □) hyperthermia (43°C, 30 min) delivered immediately after drug administration or without (●, ○) hyperthermia.

were equally augmented by combination with hyperthermia at therapeutically achievable doses.

The tumour cell survival and bone marrow CFU-GM characteristics of tetraplatin, on the other hand, were quite different than those of cisplatin [52, 53]. In the dosage range from 5 to 40 mg/kg of each drug, cisplatin killed 0.10 logs of FSaIIC tumour cells per mg/kg administered whereas tetraplatin killed 0.045 logs of FSaIIC tumour cells per mg/kg administered to the animals. Thus, a dose of 22 mg/kg of tetraplatin is required to kill 1 log of FSaIIC tumour cells while a dose of only 9.85 mg/kg cisplatin is required to kill 1 log of FSaIIC tumour cells. Tetraplatin is much more toxic toward bone marrow CFU-GM than is cisplatin—in fact at the same dose tetraplatin killed 1 log more bone marrow CFU-GM than did cisplatin [52, 53].

Tetraplatin is an interesting new platinum complex. The D, L-form of tetraplatin is currently undergoing phase I clinical trial. Our results support the notion that there are no cytotoxic, antitumour or bone marrow toxicity reasons to warrant using the pure D-isomer in the clinic. However, other toxicities may arise with the racemic mixture which have yet to be detected. Our results indicate that tetraplatin would be an appropriate drug for use in combination with local hyperthermia but perhaps not with whole-body hyperthermia unless bone marrow transplantation or growth factor cotreatment is planned.

Table 2. DNA crosslinking factors from FSaIIC tumours

		DNA crosslinking factor*		Surviving	
Treatment	Dose (mg/kg)	37°C	43°C 30 min	37°C	43°C 30 min
Cisplatin	10	1.7	4.7	0.30	0.0017
D, L-Tetraplatin	10	2.9	7.8	0.27	0.18
D-Tetraplatin	10	4.0	8.6	0.23	0.15

*A DNA crosslinking factor = 1.00 indicates no crosslinks. The tumour cell surviving fraction produced by hyperthermia (43°C, 30 min) was 0.65.

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