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Evaluation of *trans*-tetrachloro-1,2-diaminocyclohexane platinum (IV) in murine leukemia L1210 resistant and sensitive to *cis*-diamminedichloroplatinum (II)*

Lee J. Wilkoff, Elizabeth A. Dulmadge, Mary W. Trader†, Steadman D. Harrison, Jr., and Daniel P. Griswold, Jr.

Kettering-Meyer Laboratory, Southern Research Institute, P. O. Box 55305, Birmingham, AL 35255-5305, USA

Summary. *trans*-Tetrachloro-1,2-diaminocyclohexane platinum (IV) (tetraplatin) was therapeutically effective in mice bearing leukemia L1210 resistant (L1210/DDPt) or sensitive (L1210/0) to *cis*-diamminedichloroplatinum (II) (cisplatin). Furthermore, the sensitivity of cultured L1210/DDPt and L1210/0 cell populations to tetraplatin, cisplatin, and dichloro-*trans*-dihydroxyisopropylamine platinum (IV) (CHIP) was a function of the concentrations used for each compound. The relative degree of sensitivity between cultured L1210/DDPt and L1210/0 cells for each compound on the basis of the LC_{99} (the concentration of each compound required to reduce the number of viable cells by 99% in each cell line) was 3-fold for cisplatin, 2-fold for tetraplatin, and 3-fold for CHIP; thus the cultured L1210/0 cells exhibited a greater degree of sensitivity than the L1210/DDPt cells to the platinum compounds. The data indicate that if reduction of platinum IV compounds to platinum II compounds or metabolites is required for antitumor activity, then the cultured L1210 cells are capable of this bioreduction independently of any host factors.

Introduction

Cisplatin has therapeutic efficacy alone or in combination with other antitumor drugs in the treatment of a number of tumors [7, 29]. Numerous clinical trials are in progress to test both the application of this agent to various histological types of cancers and also the use of altered clinical protocols for improved therapeutic activity against responsive tumors. Platinum IV analogues of cisplatin, *cis*-dichloro-*trans*-dihydroxyisopropylamine platinum IV (CHIP) and *trans*-tetrachloro-1,2-diaminocyclohexane platinum IV (tetraplatin) are being developed as alternatives to cisplatin in an attempt to reduce host toxicity, improve formulations, or circumvent resistance to cisplatin [1, 11].

We have evaluated tetraplatin in mice bearing leukemia L1210 resistant (L1210/DDPt) or sensitive (L1210/0) to cisplatin and in cell culture populations derived from the tumors. In addition, we compared the cell culture cytotoxicity of tetraplatin with that of CHIP.

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† Mrs Mary W. Trader died on January 16, 1987

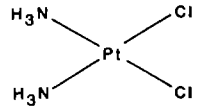
Offprint requests to: Lee J. Wilkoff

Materials and methods

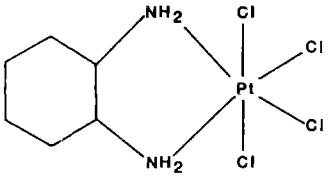
Leukemia L1210 was supplied by the Tumor Repository of the National Cancer Institute (NCI). The L1210/0 line was maintained in serial passage as described in the NCI protocols [18].

L1210/DDPt was developed by treatment of BALB/c ♀ × DBA/2 ♂ (CD2F1) mice bearing ascites tumor with cisplatin over a number of transplant generations. A total of 1×10^5 L1210 cells were implanted i. p., and mice were treated with cisplatin (3.5 mg/kg) on days 1, 5, and 9 after implantation. When one half of the mice had died, tumor cells were harvested from a survivor that exhibited a frank accumulation of ascites fluid; a series of passages was then started by i. p. injection 1×10^5 cells into other recipient mice followed by cisplatin therapy (5 mg/kg) on day 4 after implantation. Partial resistance to cisplatin developed on the first passage. At present, comparative in vivo drug studies indicate that the L1210/DDPt cell populations under optimal treatment with cisplatin will increase by 1–3 $\log_{10}$ units compared with the parent L1210/0 cell populations that undergo a 2- to 5-$\log_{10}$-unit decrease in viability under optimal therapy with cisplatin. The L1210/DDPt tumor is routinely maintained in CD2F1 mice by implantation of 1×10^5 cells per mouse, followed by treatment on the 4th day after implantation with a single suboptimal dose of cisplatin (5 mg/kg). The resistant tumor is stored at −209° C in liquid nitrogen when not in passage. When removed from storage, the cells are implanted into CD2F1 mice and maintained without treatment for one passage generation before being treated with 5 mg/kg cisplatin.

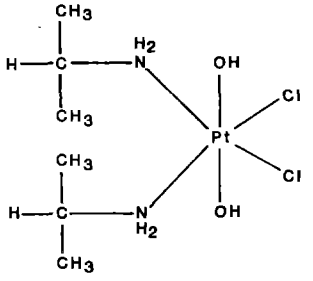
Therapeutic trials to test the in vivo antitumor activity of tetraplatin as compared with cisplatin were accomplished according to NCI protocols [18]. A titration of each inoculum from 10^7 to 10^2 cells was included in every experiment to provide data on tumor stem cell doubling time. Testing was carried out over a range of doses from frankly toxic to nontoxic and inactive dose levels in parallel groups of mice bearing the drug-resistant or parent drug-sensitive leukemias. These groups were treated with the same drug preparation. The $\log_{10}$ change in the tumor burden after the last treatment was estimated from the median survival time of treated mice relative to that of the untreated control mice, the doubling times of the tumor cell populations, and the number of drug treatments [23]. Response of the resistant tumor was compared with that of the parent sensitive tumor line at the nontoxic dose that



CISPLATIN



TETRAPLATIN



CHIP

Fig. 1. Structures of platinum compounds

showed optimal activity against the drug-sensitive tumor cells.

Proliferating cultured L1210/0 cells were derived from sensitive cell populations passaged in vivo in CD2F1 mice, and cultured L1210/DDPt cells were established from resistant cell populations developed in vivo. Stock cultures of leukemia L1210/0 and L1210/DDPt were propagated in Dulbecco's modified Eagle's minimum essential medium (DMEMEM, Flow Laboratories, Inc., Rockville, Md, USA) [15] supplemented with 10% horse serum (Flow Laboratories) and 5 μM 2-mercaptoethanol [8]. The cell lines were maintained in suspension cultures as previously described [28].

The effect of the platinum compounds on the viability of proliferating cultured cell populations was determined using procedures previously described [27]. Proliferating cell populations were exposed to selected drug concentrations for 4.5 h, and the number of surviving cells was determined by colony formation in semisolid medium [27]. Colony formation efficiencies of control cultures were 43%±16% (mean ± SD) for L1210/0 and 59%±11% for L1210/DDPt. We selected an exposure period of 4.5 h since this period is equivalent to three half-lives of cisplatin in serum at 37° C [20].

In the design of the drug studies, the in vitro concentrations for the platinum compounds were based on the in vivo LD_{10} data in order to estimate pharmacologically acceptable levels of drug in vivo [22]. The cell culture equivalent dose (CCED) is defined as $1.3 \times LD_{10}$ [26] and is used as an estimate of the maximum drug concentration acceptable in vivo.

When the sensitivities of L1210/DDPt and L1210/0 cell populations to the platinum compounds were compared, the two cell populations were evaluated in parallel in the same experiment. Surviving fractions for each cell population at a specific concentration were compared by use of Chi-square analysis.

Cisplatin, tetraplatin, and CHIP (Fig. 1) were obtained from the Developmental Therapeutics Program, Division of Cancer Treatment, NCI. The platinum compounds were dissolved in a minimum amount of sterile saline at 25° C and diluted to appropriate final concentrations with cell culture medium.

Results

Tetraplatin is therapeutically effective in the treatment of CD2F1 mice bearing i. p. implants of 1×10^5 L1210/DDPt cells (Table 1). Optimal treatment ($\leq LD_{10}$) on days 1, 5, and 9 produced in vivo cell kill of 6 $\log_{10}$ units in mice bearing L1210/DDPt and in mice bearing L1210/0 cells.

Table 1. Comparative therapeutic effect of tetraplatin and cisplatin in leukemic CD2F1 mice bearing either L1210/DDPt or L1210/0 tumors

Drug	mg/kg per dose	Schedule	L1210/0[a]			L1210/DDPt[a]		
			30-Day survivors	% ILS	Approx. $\log_{10}$ change in tumor burden after last Rx[b]	30-Day survivors	% ILS	Approx. $\log_{10}$ change in tumor burden after last Rx[b]
Cisplatin	20	Days 1, 5, 9	0/6	+ 48	Toxic[c]	0/6	− 14	+3
	10	Days 1, 5, 9	3/6	+248	−6	0/6	+ 8	+3
	5	Days 1, 5, 9	0/6	+ 62	+2	0/6	+ 1	+3
Tetraplatin	16	Days 1, 5, 9	1/6	+ 43	Toxic[c]	1/6	+ 10	Toxic[c]
	8	Days 1, 5, 9	3/6	+248	−6	4/6	+222	−6
	4	Days 1, 5, 9	2/6	+112	−2	2/6	+125	−3

[a] Implant: i. p. 1×10^5 cells

[b] Based on median survival time; in groups of mice having 50% more animals alive at 30 days, the $\log_{10}$ cell kill is based on the number of surviving mice [5]

[c] More than 16% of the deaths were drug-related based on the absence of ascitic fluid and/or splenomegaly at necropsy examination

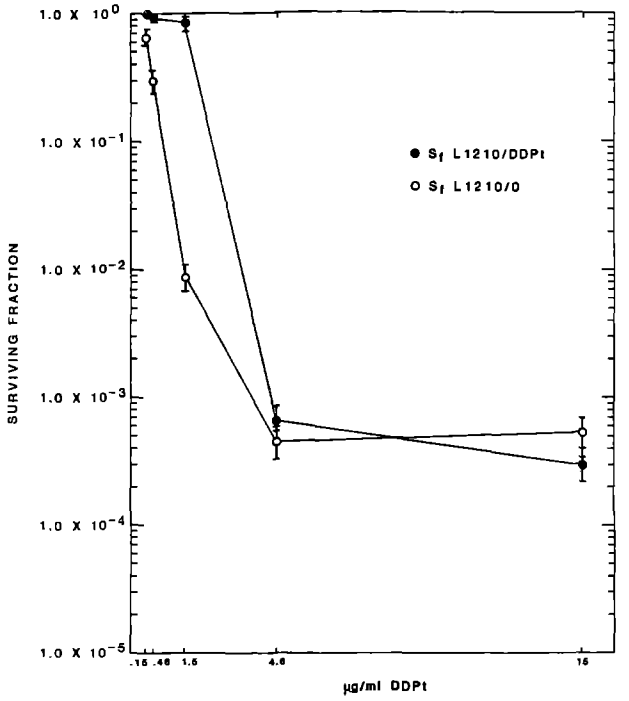


Fig. 2. Sensitivity of cultured L1210/DDPt and L1210/0 cells to cisplatin (0.15–15 µg/ml) for a 4.5-h exposure period. Surviving fractions for each cell population were compared by use of Chi-square analysis. Differences in surviving fractions were significant ($P < 0.001$) at 0.15, 0.46, and 1.5 µg/ml DDPt. *Bars*, 95% confidence limits

In contrast, optimal treatment with cisplatin ($\leq LD_{10}$) on days 1, 5, and 9 produced in vivo cell kill of 6 $\log_{10}$ units in mice bearing L1210/0 cells, but L1210/DDPt cells actually increased by 3 $\log_{10}$ units under treatment.

Proliferating cultured L1210/DDPt cell populations were resistant to 0.15, 0.46, and 1.5 µg/ml cisplatin compared with the L1210/0 cell populations (Fig. 2). We could not demonstrate resistance to 4.6 or 15 µg/ml cisplatin (Fig. 2). The data indicated that the degree of resistance to this agent was a function of the drug concentration employed.

Cultured populations of L1210/DDPt cells were sensitive to tetraplatin and exhibited a dose response in the concentration range used (Fig. 3). A concentration of 2.5 µg/ml reduced the number of viable cells in the population by approximately 2 $\log_{10}$ units, and 8.1 µg/ml reduced the viability of the cell population by about 3 $\log_{10}$ units.

Populations of cultured L1210/DDPt cells were also sensitive to CHIP (Fig. 4). Concentrations of 23.5 and 75.0 µg/ml reduced the viability of the cell populations by about 2 and 3 $\log_{10}$ units, respectively. There was no difference in sensitivity between L1210/DDPt and L1210/0 cells exposed to 75 µg/ml CHIP.

On a molar basis, tetraplatin was more effective than CHIP and cisplatin in reducing the number of viable cells in the L1210/DDPt cell populations. CHIP and cisplatin exhibited comparable activity in reducing the viability of the L1210/DDPt cell populations. The relative degree of sensitivity between L1210/DDPt and L1210/0 cells for

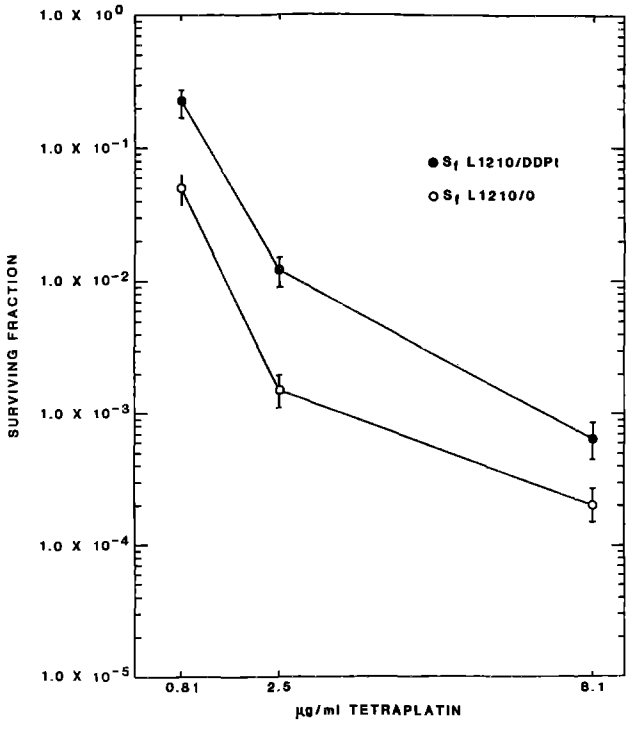


Fig. 3. Sensitivity of cultured L1210/DDPt and L1210/0 cells to tetraplatin (0.81–8.1 µg/ml) for a 4.5-h exposure period. Surviving fractions for each cell population were compared by use of Chi-square analysis. Differences in surviving fractions were significant ($P < 0.001$) at 0.81, 2.53 and 8.1 µg/ml tetraplatin. *Bars*, 95% confidence limits

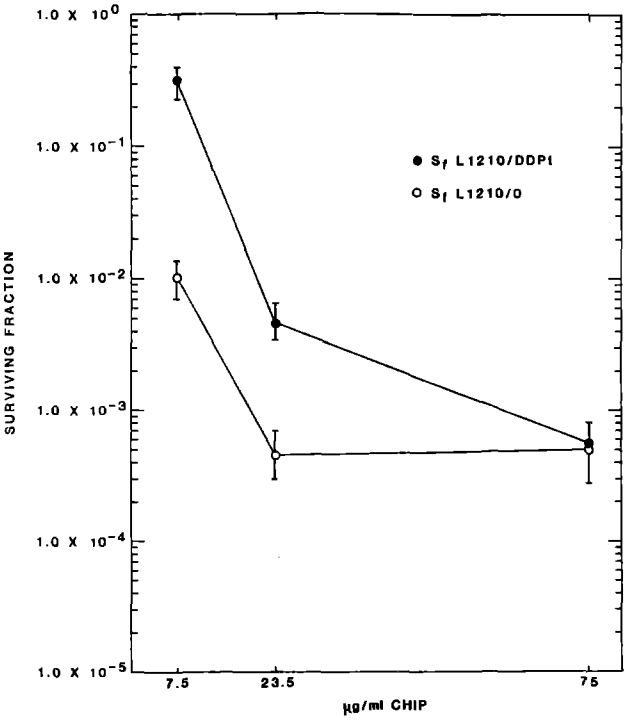


Fig. 4. Sensitivity of cultured L1210/DDPt and L1210/0 cells to *cis*-dichloro-*trans*-dihydroxyisopropylamine platinum (IV) (CHIP; 7.5, 23.5, 75 µg/ml) for a 4.5-h exposure period. Surviving fractions for each cell population were compared by use of Chi-square analysis. Differences in surviving fractions were significant ($P < 0.001$) at 7.5 and 23.5 µg/ml CHIP. Bars, 95% confidence limits

each compound on the basis of the LC_{99} concentrations estimated from the data in Fig. 2–4 showed a 3-fold difference for cisplatin, a 2-fold difference for tetraplatin, and a 3-fold difference for CHIP, indicating that cultured L1210/0 cell populations were more sensitive than the L1210/DDPt cell populations to the platinum compounds.

Discussion

Tetraplatin, a new platinum IV analogue under development, is currently undergoing preclinical toxicity evaluation and has been selected for clinical development [12–14, 19, 24]. CHIP is also a platinum IV analogue that was developed as an alternative to cisplatin [7, 29]. CHIP may act as a prodrug of a platinum II complex, as a platinum IV complex, or as a combination of both these complexes [10].

Burchenal [9] has reported that several platinum congeners with 1,2-diaminocyclohexane substitutions have exhibited no cross-resistance to leukemias P388 or L1210 that are resistant to cisplatin. We report here the observation that tetraplatin, a platinum IV congener with a 1,2-diaminocyclohexane substitution, is therapeutically effective against mice bearing L1210/DDPt or L1210/0 leukemia, which confirms the previously reported results of Anderson et al. [1]. This congener is also therapeutically active in mice bearing P388/DDPt or P388/0 leukemia (unpublished data and [1]). Treatment data with CHIP on mice bearing L1210/DDPt leukemia show cross-resistance to CHIP (W. C. Rose, personal communication 1986; [21]). Also, in mice bearing L1210/0 leukemia, CHIP and cisplatin show approximately the same activity [29]. In addition, cultured L1210/DDPt cell populations are sensitive to tetraplatin at estimated pharmacologically acceptable concentrations, although they are less sensitive than cultured L1210/0 cell populations. The cultured L1210/DDPt cells also exhibit sensitivity to CHIP, a platinum IV congener with a diisopropylamine substitution. Therefore, at least in the L1210 system, the cytotoxic effects of the platinum IV congeners on tumor cells appear to be independent of any host factors. CHIP has also been reported to produce the following cytotoxic effects in cell culture: chromosomal aberrations [5], sister chromatid exchanges [6], single-strand breaks [5], and perturbation of the cell cycle [2]. However, a cultured human ovarian cancer cell line resistant to cisplatin [16] has been reported to be resistant to tetraplatin and CHIP [3].

It has been postulated that since platinum IV complexes are exchange-inert [4], binding to DNA in a manner similar to that of cisplatin may not be the basis for the cytotoxicity of CHIP or tetraplatin. Tobe and Khokhar [25] and Cleare et al. [10] have suggested that platinum IV analogues may be activated in vivo by reduction to platinum II compounds, which then exert their cytotoxic effects in a manner analogous to cisplatin. Significant in this regard was the observation that one of the metabolites of CHIP, recovered from the plasma and urine of cancer patients receiving the drug [17], was the platinum II compound *cis*-dichlorobis(isopropylamine) platinum II.

The data in this study indicate that tetraplatin is cytotoxic for L1210/DDPt or L1210/0 cells both in vivo and in cell culture. Although CHIP is cytotoxic to cultured L1210/DDPt cells, the cytotoxic potency is reduced from that observed in cultured L1210/0 cells and the degree of cell killing in L1210/DDPt cell populations is comparable to that observed using cisplatin. Therefore, if reduction of platinum IV compounds to platinum II compounds or platinum II metabolites is required for antitumor activity, the data demonstrate that L1210 cell populations are capable of this reduction independent of any host factors.

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