

Brief Communication

Lack of Cross-resistance between Certain Platinum Coordination Compounds in Mouse Leukemia¹

Joseph H. Burchenal, Kathleen Kalaher, Tara O'Toole, and Joan Chisholm

Memorial Sloan-Kettering Cancer Center, New York, New York 10021

SUMMARY

Two congeners of *cis*-platinum diamminodichloride, 1,2-diamminocyclohexylplatinum malonate (NSC 224964) and 1,2-cyclohexyldiamminoplatinum sulfate (NSC 250427), show approximately equal inhibitory activity *in vitro* against leukemia L1210 and a line of L1210 (L1210/PDD) that has developed resistance to *cis*-platinum diamminodichloride. These compounds are also active against L1210/PDD *in vivo*. These observations suggest that they be tried clinically in patients whose disease has become resistant to *cis*-platinum diamminodichloride.

INTRODUCTION

The discovery by Rosenberg *et al.* (15, 17) of the effect of PDD² on the inhibition of microbial division led to studies of its effects on the growth of mouse leukemias and solid tumors (18). The activity in these systems (5, 11, 16, 21) led to clinical trials (9) and the demonstration of significant clinical activity against testicular tumors (3, 6, 13), tumors of the head and neck (6, 14), and bladder carcinoma (22). At first, the compound was limited in the amount that could be given because of renal toxicity, but the fundamental studies (first in dogs and then in humans) of Hayes *et al.* (7) demonstrated that, when the kidneys were protected by mannitol diuresis, 3-fold higher doses (120 mg/sq m) could be given without significant renal impairment. Similar protection by diuresis against smaller weekly doses has also been shown by Merrin (13). Many derivatives of PDD with antineoplastic activity have been synthesized, and some (at least in plasma cell tumors, but not in L1210 leukemia) seem to have a much higher chemotherapeutic index than PDD itself (2). Two compounds, 1,2-diamminocyclohexylplatinum malonate (NSC 224964) and 1,2-diamminocyclohexylplatinum sulfate (NSC 250427), have been reported recently by Speer *et al.* (19, 20) to be highly active against mouse leukemias and by Hill *et al.* (8) and Loeb *et al.* (12) to have clinical activity against acute myeloblastic leukemia or to possess activity against some carcinomas. For this reason we have

been interested in studying these compounds in various mouse leukemias and particularly both *in vitro* and *in vivo* in a line of leukemia L1210 selected for resistance to PDD (L1210/PDD).

MATERIALS AND METHODS

The technique for evaluating the chemotherapeutic activity of a drug by its ability to prolong the survival time of mice with transplanted leukemias has been reported previously (1). The experiments described here were done with mouse leukemia L1210 (10) and its PDD-resistant subline, L1210/PDD, in C57BL × DBA/2 F₁ mice. The resistant subline, L1210/PDD, is a line of L1210/0 that has been treated with single doses of PDD (8 mg/kg on Day 1) over successive generations until no increase in survival time is seen with any tolerated dose of PDD. Generations 255 to 262 were used in these experiments. One million leukemic cells suspended in 0.85% NaCl solution were inoculated i.p. into each animal, producing an ascitic leukemia that later progressed to the generalized disease. The mice were divided into groups of 10 animals each, and treatment was initiated 24 hr after the inoculation of leukemic cells and continued once every 4th day for 4 doses. Compounds were dissolved in 0.85% NaCl solution and injected i.p.

For cell culture studies, a modification (1) of the technique of Fischer (4) was used. The cells were incubated in McCoy's medium with 15% fetal calf serum. The initial inoculum was 40,000 to 60,000 leukemic cells/ml. For growth inhibition studies, 0.1 ml of a 50-fold concentration of the drug in question was added to 5 ml of the cell-containing medium. The tubes were set up in groups of 4, loosely capped, and allowed to incubate in 5% CO₂ at 37° for 96 hr. Growth to approximately 10⁶ cells/ml occurred in the control tubes. The contents of each tube were agitated to suspend the cells and counted on a Coulter counter. The percentage of growth inhibition and ID₅₀ values were calculated. Cell culture experiments were done with mouse leukemia cell lines L5178Y, L1210, and the PDD-resistant L1210/PDD.

RESULTS

As can be seen in Table 1, the line of mouse leukemia L1210/PDD resistant *in vivo* to PDD showed a 30- to 80-fold

¹ Supported by Grants 05826, 07848, and 18856 from the National Cancer Institute; The American Cancer Society Laurens Hammond Memorial CH-27S; and the Hearst Foundation.

² The abbreviations used are: PDD, *cis*-platinum diamminodichloride; ID₅₀, drug concentration that reduced cell count at 96 hr after initiation to 50% of value for untreated control.

Received June 15, 1977; accepted July 6, 1977.

increase in resistance when it was tested quantitatively *in vitro*, with an ID₅₀ of 0.05 µg/ml in the sensitive parent line, compared to an ID₅₀ in the resistant line of 1.2 to 4.7 µg/ml. As Table 1 also shows, however, the ID₅₀'s for both the malonate (NSC 224964) (0.2 µg/ml) and the sulfate (NSC 250427) (0.2 µg/ml) derivatives were roughly similar for both the parent L1210 and the PDD-resistant L1210/PDD.

Table 1
ID₅₀'s of the 3 platinum derivatives (µg/ml) against PDD-sensitive and -resistant lines

Drug	L5178Y	L1210	L1210/PDD
NSC 224964 (malonato)	0.5	0.2	0.5
		0.2	0.5
			0.2
			0.1
NSC 250427 (sulfato)	0.4	0.2	0.5
			0.6
		0.2	0.2
			0.19
PDD	0.06	0.06	4.7
			2.1
		0.03	1.7
			1.1

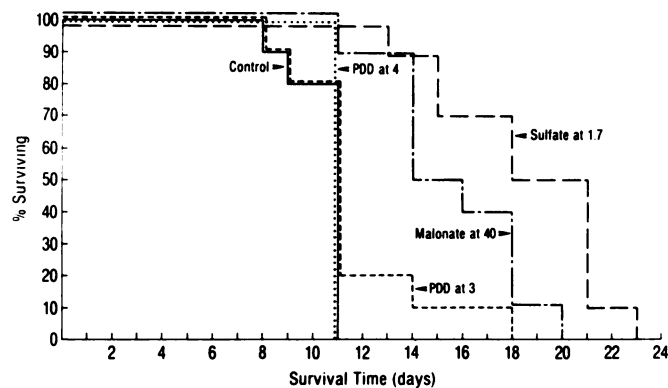


Chart 1. The effects of PDD compared to the 1,2-diamminocyclohexylplatinum malonate and sulfate compounds in leukemia L1210/PDD; doses in mg/kg every 4 days for 4 doses.

These experiments have been repeated several times with generally consistent results. As can be seen in Chart 1, PDD at the usual dose of 3 to 4.0 mg/kg every 4th day for 4 doses had no effect in prolonging the survival time of mice inoculated with 10⁶ cells of L1210/PDD. As mentioned in "Materials and Methods," this is a line that has been exposed to treatment with PDD for over 250 generations *in vivo*, 8 mg/kg on Day 1. As can be seen in Chart 1 and Table 2, however, both the sulfate and the malonate at doses that do not cause lethal toxicity produce a definite increase in survival time, although the increase is somewhat less than that in the sensitive L1210.

DISCUSSION

The mechanism of this lack of cross-resistance is not understood at the present time. Other congeners with substituted diammino groups of platinum(II) or platinum(IV) with chlorine instead of the malonate or sulfate groups have shown cross-resistance in this line. These data and *in vitro* studies with 2 other substituted diammino platinum derivatives containing a sulfate or a phosphate group in place of the 2 chlorines, which have indicated a relative lack of cross-resistance, suggest that the substitution of the bidentate SO₄, PO₄, or malonate for the Cl₂ may be responsible for this lack of cross-resistance. We thus far have not had the opportunity to test other unsubstituted diammino congeners with sulfate or malonate groups against this line of L1210/PDD. Whether the activity of the sulfate (NSC 250427) and the malonate (NSC 224964) derivatives will also hold for other lines of leukemia or tumors developing resistance to PDD remains to be seen. We know of no other available PDD-resistant lines of mouse leukemia, but we are presently attempting to develop other lines. The facts that in this line, L1210/PDD, which has developed 30- to 80-fold resistance to PDD, there is no significant increase in resistance *in vitro* to either the malonate or the sulfate compound and that in L1210/PDD *in vivo* the compounds still have a very definite therapeutic effect suggest that at least 1 of these compounds should be subjected to Phase 1 and 2

Table 2
Effects of 3 platinum derivatives on survival times in PDD-sensitive and -resistant leukemia L1210

Compound	Dose (mg/kg)	L1210/0 ^a		L1210/PDD ^b	
		Survival time (days)	% increase in life-span	Survival time (days)	% increase in (days)
Control		8.6 ± 1.3 ^c		10.8 ± 3.5 ^c	
PDD	6.75			10.9 ± 0.10	
PDD	4.50			10.1 ± 5.1	9
PDD	3	17.0 ± 5.3	98	11.7 ± 2.2	8
Malonate (NSC 224964)	40	22.9 ± 6.2	166	20.8 ± 2.1	92
Sulfate (NSC 250427)	6.75			16.0 ± 4.5	48
Sulfate (NSC 250427)	4.5			18.2 ± 2.7	69
Sulfate (NSC 250427)	3.3	19.0 ± 7.9	120		
Sulfate (NSC 250427)	3.0			14.5 ± 5.3	34
Sulfate (NSC 250427)	2.0			16.8 ± 4.1	56
Sulfate (NSC 250427)	1.33			16.5 ± 4.6	53

^a Exp. 6832.

^b Exp. 6933.

^c Mean ± S.D.

trials in patients, particularly those patients with germ cell tumors that have become resistant to PDD.

REFERENCES

- Burchenal, J. H., Adams, H. H., Newell, N. S., and Fox, J. J. Comparative Activity of 1- β -D-Arabinofuranosyl-5-fluorocytosine and Related Compounds against Transplanted Mouse Leukemias *in Vivo* and *in Vitro*. *Cancer Res.*, **26**: 370-373, 1966.
- Connors, T. A., Jones, M., Ross, W. C., Bradlock, P. D., Khokhar, A. R., and Tobe, M. L. New Platinum Complexes with Antitumor Activity. *Chem.-Biol. Interactions*, **5**: 415-424, 1972.
- Einhorn, L. H., and Furnas, B. Improved Chemotherapy in Disseminated Testicular Cancer. *J. Clin. Hematol. Oncol.*, **7**: 662-671, 1977.
- Fischer, G. A. Studies of the Culture of Leukemic Cells *in Vitro*. *Ann. N.Y. Acad. Sci.*, **76**: 673-680, 1958.
- Gale, C. R., Rosenblum, M. G., Atkins, L. M., Walker, E. M., Smith, A. B., and Meischen, S. J. Antitumor Action of *cis*-Dichlorobis-(methylamine)platinum (II). *J. Natl. Cancer Inst.*, **51**: 1227-1234, 1973.
- Gottlieb, J. A., and Drewinko, B. Review of the Current Clinical Status of Platinum Coordination Complexes in Cancer Chemotherapy. *Cancer Chemotherapy Rept.*, **59**: 621-628, 1975.
- Hayes, D., Cvitkovic, E., Golbey, R., Scheiner, E., and Krakoff, I. H. Amelioration of Renal Toxicity of High-Dose *cis*-Platinum Diammine Dichloride (*c*-PDD) by Mannitol-induced diuresis. *Proc. Am. Assoc. Cancer Res.*, **17**: 296, 1976.
- Hill, J. M., Loeb, E., Pardue, A. S., Hill, N. O., Khan, A., and King, J. J. Platinum Coordination Compounds in the Treatment of Acute Leukemia and Other Malignant Diseases with Particular Reference to Malonato 2,2-Diaminocyclohexane Platinum(II). *J. Clin. Hematol. Oncol.*, **7**: 681-700, 1977.
- Hill, J. M., Speer, R. J., Loeb, E., *et al.* Clinical Experience with *cis*-platinous diamminodichloride (PDD). *Advan. Antimicrobial Antineoplastic Chemotherapy*, **2**: 255, 1972.
- Law, L. W., Dunn, T. B., Boyle, P. J., and Miller, J. H. Observations of the Effect of a Folic Acid Antagonist on Transplantable Lymphoid Leukemias in Mice. *J. Natl. Cancer Inst.*, **10**: 179, 1949.
- Leonard, B. J., Eccleston, E., Jones, D., Todd, P., and Walpole, A. Antileukemic and Nephrotoxic Properties of Platinum Compounds. *Nature*, **234**: 43-45, 1971.
- Loeb, E., Hill, J. M., Pardue, A. S., Hill, N. O., Khan, A., and King, J. J. Solid Tumor Experience with Newer Platinum Coordination Compounds. *J. Clin. Hematol. Oncol.*, **7**: 701-709, 1977.
- Merrin, C. A New Method to Prevent Toxicity with High Doses of *cis*-Diammine Platinum (Therapeutic Efficacy in Previously Treated Widespread and Recurrent Testicular Tumors). *Proc. Am. Assoc. Cancer Res.*, **17**: 243, 1976.
- Randolph, V. L., Vallejo, A., Strong, E. W., and Wittes, R. E. Combination Treatment with Chemotherapy and Radiotherapy in Head and Neck Cancer. *Proc. Am. Assoc. Cancer Res.*, **18**: 336, 1977.
- Rosenberg, B., Renshaw, E., Van Camp, L., Hartwick, D., and Drobnik, J. Platinum Induced Filamentous Growth in *Escherichia coli*. *J. Bacteriol.*, **93**: 716-721, 1967.
- Rosenberg, B., and Van Camp, L. The Successful Regression of Large Solid Sarcoma 180 Tumors by Platinum Compounds. *Cancer Res.*, **30**: 1799-1802, 1970.
- Rosenberg, B., Van Camp, L., and Krigas, T. Inhibition of Cell Division in *Escherichia coli* by Electrolysis Products from a Platinum Electrode. *Nature*, **205**: 698-699, 1965.
- Rosenberg, B., Van Camp, L., Trosko, J. E., and Mansour, V. H. Platinum Compounds: A New Class of Potent Antitumor Agents. *Nature*, **222**: 385, 1969.
- Speer, R. J., Ridgway, H., Hall, L. M., Newman, A. D., Howe, K. E., Stewart, D. P., Edwards, G. R., and Hill, J. M. Malonato 1,2-diamminocyclohexane Platinum (II), a Potential Antitumor Agent. *Wadley Med. Bull.*, **5**: 335-348, 1975.
- Speer, R. J., Ridgway, H., Stewart, H., Stewart, D. P., Hall, L. M., Zapata, A., and Hill, J. M. Sulfato 1,2-Diaminocyclohexane Platinum (II): A Potential New Antitumor Agent. *Wadley Med. Bull.*, **7**: 210-219, 1977.
- Talley, R. W. Chemotherapy of Mouse Reticulum Cell Sarcoma with Platinum Salts. *Proc. Am. Assoc. Cancer Res.*, **11**: 78, 1970.
- Yagoda, A., Watson, R., Grabstald, H., and Whitmore, W. F. *cis*-Platinum (II) Diammine Dichloride (CPDD) in Advanced Urinary Tract Cancer. *Proc. Am. Assoc. Cancer Res.*, **17**: 296, 1976.