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Effects of Dinitrato(1,2-diaminocyclohexane)platinum (NSC 239851) on Murine Myeloma and Hemopoietic Precursor Cells[1]

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SUMMARY

We studied the effects of dinitrato(1,2-diaminocyclohexane)platinum (NSC 239851) on murine myeloma Adj. PC-5 and hemopoietic precursor cells. The median survival time of tumor-bearing mice was significantly affected by i.p. injections of this agent. While 14 mg/kg was a toxic dose, as little as 2 mg/kg prolonged the survival for over 100 days. When cells from normal marrow and actively regenerating marrows were exposed to the agent in culture tubes, washed, and assayed for the surviving hemopoietic precursors, similar sensitivity curves were observed. This result indicates the absence of cell cycle dependency of the agent. When marrow cells were exposed to NSC 239851 at 4° in culture, almost total abrogation of the cytotoxicity was noted. This observation, unlike that from the experiment with the inorganic platinum congener, *cis*-diamminedichloroplatinum, suggests that the transport of this agent is an active process.

INTRODUCTION

Platinum compounds appear to constitute a new class of effective antineoplastic agents. Many platinum congeners are being synthesized and studied for their potential effectiveness in the treatment of experimental neoplasms, and some of them have demonstrated significant antitumor activity against murine tumors (5). One of the original compounds, *cis*-diamminedichloroplatinum (NSC 119875), is now available for phase II clinical evaluation, and preliminary data suggest that this agent is effective against some of the human solid tumors (7). Its severe nephrotoxicity, however, does not permit repeated administration to patients. Recently, Gale and Meischen synthesized dinitrato(1,2-diaminocyclohexane)platinum (NSC 239851) and, upon testing against L1210 leukemia, discovered significant antitumor activity. When 0.5 mg/kg was given to C57BL/6 × DBA/2 mice on Days 1 through 9 following administration of 10^5 L1210 cells on Day 0, the mean life-span was increased 281% and 30% of the mice were cured. This compound is more soluble in water than NSC 119875 and appears to be significantly less nephrotoxic than NSC 119875 (unpublished data). The solubility of NSC 239851 in 5% glucose water is 3 mg/ml while that of NSC 119875 is 1 mg/ml.

Murine myeloma, Adj. PC-5, carried in BALB/c mice is an excellent model for the chemotherapy of human myeloma. This tumor is highly sensitive to melphalan and cyclophosphamide, while being moderately sensitive to 1,3-*bis* (2-chloroethyl)-1-nitrosourea and completely insensitive to 5-fluorouracil (8, 9). In this report, we describe studies of the effects of NSC 239851 on Adj. PC-5 and hemopoietic precursor cells. The compound in a small dosage was successful in eradicating the tumor cells. Results further suggested that the agent has no cell cycle specificity.

MATERIALS AND METHODS

Mice. BALB/c mice were obtained through Mammalian Genetics and Animal Production Section, Drug Research and Development, National Cancer Institute. Mice weighing 20 to 25 g were used for transfers of myeloma and studies of the sensitivity of hemopoietic precursor cells.

NSC 239851. To 1.0 g of dichloro(1,2-diaminocyclohexane)platinum(II) (NSC 194814) was added 0.88 g of $AgNO_3$ in 10 to 20 ml of distilled water. This was stirred for at least 3 hr at room temperature. The insoluble AgCl was removed by centrifugation followed by filtration of the supernatant solution. The volume of filtrate was reduced by passing a stream of filtered air over it, and the reduced volume was placed in a vacuum desiccator to dry completely. The residue was scraped from the beaker, taken up in a minimal volume of water, and filtered; the product on the filter paper was washed with a small volume of dilute HNO_3 followed by a small volume of water. The product was finally dried under reduced pressure over fuming sulfuric acid. The yield was 55%. Elemental analyses agreed with theoretical values within 0.2%. The compound was initially dissolved in 5% glucose solution, and the subsequent dilution was made in 0.9% NaCl solution.

Mouse Myeloma (Adj. PC-5). This myeloma was obtained in s.c. form from Litton Bionetics, Kensington, Md. The tumor was transferred fortnightly by transplanting a small fragment with a 15-gauge needle. Experiments were carried out with cells from transplant generations 119 to 131.

Survival Studies. Effect of NSC 239851 on Adj. PC-5 was examined *in vivo* through survival studies and compared

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with that of NSC 119875. Synthesis of the latter compound has been described previously (11). A small piece of tumor was transplanted s.c. as described above. Ten days later, groups of mice received a single i.p. injection of varying doses of NSC 239851 and NSC 119875. Survival of the mice was checked daily, and the median survival times were calculated.

Culture Assay for Murine Hemopoietic Precursor Cells. Bone marrow cells were obtained from femurs of BALB/c mice as described previously (13). Marrow cells capable of forming granulocytic CFU-C[3] were assayed by modification of the technique described by Worton *et al.* (14). α medium (Flow Laboratories, Inc., Rockville, Md.) was used instead of Connaught Medical Research Laboratories Medium 1066 (Connaught Laboratories, Ltd., Willowdale, Ontario, Canada), and bovine serum albumin was omitted. Marrow cells in single-cell suspensions were immobilized in 0.8% methylcellulose (Fisher Scientific Co., Norcross, Ga.), 20% fetal bovine serum (Flow Laboratories), 20% conditioned medium from murine L-cell monolayers, and α medium. Incubation was carried out at 37° in a humidified 5% CO_2 in air atmosphere for 7 days, and the enumeration of colonies was done on an inverted microscope. Cells thus plated yielded approximately 150 colonies/10^5 nucleated cells (11).

Cells from Regenerating Bone Marrow. Mice with regenerating bone marrow were prepared by the i.v. injection of 1×10^7 nucleated marrow cells into lethally irradiated isologous animals. They had received 850 rads of total body radiation using the 250-kVp GE Maxitron unit at 30 ma and 0.25 copper filtration. The treatment distance was 50 cm and no cone was used. The machine calibration was checked immediately prior to the radiation procedure using a Victoreen condenser R meter 570, probe 651. The femoral marrow of these mice was harvested 6 days later.

Exposure to NSC 239851. For dose-response studies, murine bone marrow cells at a concentration of 10^6 nucleated cells/ml were incubated with varying concentrations of NSC 239851 for 1 hr at 37° in 16-ml Falcon plastic tissue culture tubes. In temperature-response studies, marrow cells in concentrations of 10^6 nucleated cells/ml were exposed to NSC 239851, 3.0 μg, for 1 hr at 4, 24, and 37°. Following incubation, cells were washed twice with cold α medium and were plated in culture in quadruplicate at a final concentration of 1×10^5 cells/ml. The mean ± S.E. of the quadruplicate data was calculated using a Wang 600 programmable calculator and the results were expressed as percentage of survival of CFU-C of the drug-treated groups. The relevance of this test system in the evaluation of chemotherapeutic agents has been presented in previous publications (8, 9).

RESULTS

Effects of the Platinum Compounds on Murine Myeloma (Adj. PC-5) *in Vivo*. Table 1 presents the results of an experiment in which groups of 9 to 10 mice received varying doses of NSC 239851 and NSC 119875 10 days after tumor transplantation. Doses of NSC 239851 between 2 and 10 mg/kg yielded survival in excess of 100 days. Mice receiving 14 mg/kg died of toxicity a few days after the drug injection. NSC 119875 was slightly more toxic and a 10-mg/kg dose was lethal. Doses between 2 and 7 mg/kg, however, yielded median survival exceeding 100 days. The median survival time of control mice was 20 days following tumor transplantation.

Cell Cycle Dependency. Marrow cells that are active in cell proliferation have been shown to be more sensitive to such chemotherapeutic agents as vinblastine and 5-fluorouracil than those from steady-state bone marrow both *in vivo* (3) and in culture (8, 9). Cells from both normal and regenerating marrow were exposed to increasing concentrations of NSC 239851 in culture (see "Materials and Methods") and were assayed at a concentration of 1×10^6 nucleated cells/ml for surviving CFU-C. The means and S.E. of 4 experiments are presented in Chart 1. The results clearly showed that the sensitivity of marrow granulocytic precursors to this agent is not influenced by their proliferative state.

Effects of Temperature. Membrane transport of this compound by the granulocytic precursors was studied indirectly using gradients of temperature. It has been shown that active transport (6) and murine CFU-C cytotoxicity (10) of nitrogen mustard are temperature dependent. Murine marrow cells were exposed to NSC 239851 in concentrations of 3 μg/ml at various temperatures for 1 hr, washed twice, and assayed for the surviving CFU-C. This concentration of the agent would reduce the survival of CFU-C to 5% of control at 37° (Chart 1). Significant temperature dependence of the cytotoxicity of this compound is presented in Chart 2. While NSC 239851 revealed 95% killing at 37°, almost total abrogation of the cytotoxicity of this compound was observed at 4°.

DISCUSSION

Effective chemotherapy in myeloma is manifested by the disappearance of symptoms and is associated with significant prolongation of survival. Unfortunately, such response is seen only in about one-half of the patients treated with melphalan or cyclophosphamide (12). Discovery of additional effective chemotherapeutic agents for myeloma is strongly needed.

Platinum congeners may prove promising for the treat-

Table 1

Median survival time of tumor-bearing mice treated with various doses of NSC 239851 and NSC 119875[a]

Dose (mg/kg)	Median survival time	
	NSC 239851	NSC 119875
0	20	
2	>100 (6/10)[b]	>100 (6/9)
4	>100 (8/10)	>100 (7/9)
8	>100 (7/10)	>100 (8/9)
10	>100 (8/10)	16
14	15	

[a] Recorded as days after tumor transplantation.

[b] Numbers in parentheses, number of 100-day survivors/total number of mice in a group.

[3] The abbreviation used is: CFU-C, colony-forming units in culture.

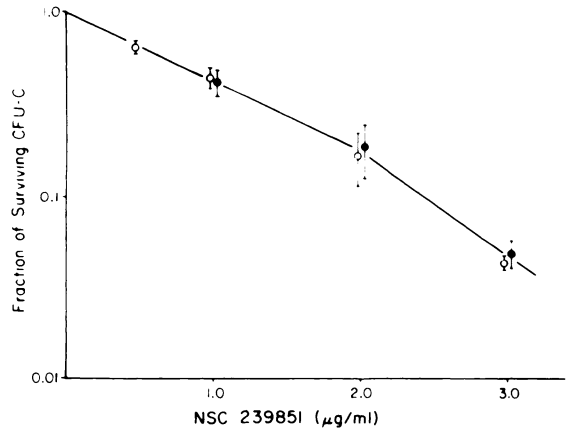

Chart 1. Sensitivity of normal and regenerating murine marrow cells to NSC 239851 in culture. Cells were exposed to concentrations of the agent for 1 hr at 37° in culture, washed twice, and assayed for the surviving fraction of CFU-C. ○, normal marrow; ●, regenerating marrow cells. *Symbols* and *bars*, mean ± S.E. of 4 experiments consisting of 4 plates.

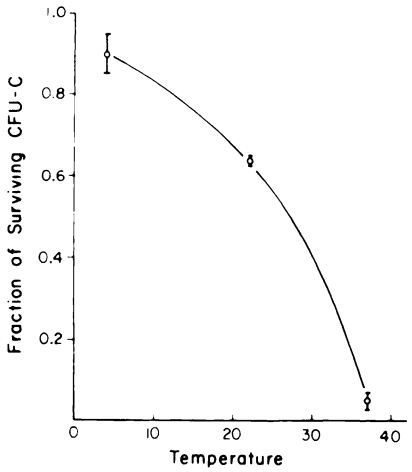

Chart 2. Sensitivity of murine CFU-C to NSC 239851 at various temperatures. Marrow cells were exposed to this agent (3.0 μg/ml) at 4, 24, and 37° for 1 hr; washed; and assayed for the surviving fraction of CFU-C. *Symbols* and *bars*, mean ± S.E. of 4 experiments.

ment of multiple myeloma. The mechanism of action of these compounds appears to be similar to that of alkylation (5), and their cell cycle independence has been demonstrated (4, 11). Currently, *cis*-diamminedichloroplatinum (NSC 119875) is being evaluated for phase II studies for its effectiveness against human multiple myeloma by Ogawa. Unfortunately, this compound possesses severe cumulative nephrotoxicity, and repeated injection is not well tolerated by patients. NSC 239851, studied in this publication, has an advantage over the former in that the nephrotoxicity appears to be minimal. Accordingly, we investigated the effect of NSC 239851 on a model murine plasmacytoma, Adj. PC-5, and compared it with that of NSC 119875. Both compounds, in relatively small doses, were effective in eradicating the tumor, suggesting their selective toxic effects on the tumor cells. We have not determined the minimal effective doses for these compounds. Accurate quantitation of the therapeutic indices of these compounds, however, must be carried out in culture (9).

Bruce *et al.* (3) presented data showing that actively regenerating hemopoietic stem cells are more sensitive to such chemotherapeutic agents as vinblastine or 5-fluorouracil than those from steady-state marrow, and they provided rationale for the current high-dose intermittent chemotherapy (2). The fraction of marrow stem cells in cell cycle is larger in regenerating marrow than in steady-state marrow (1). It is generally accepted that these chemotherapeutic agents act preferentially on cells in specific phases of a cell cycle and thus are more toxic to the former than to the latter. Ogawa has previously shown that cell culture studies of normal and regenerating murine marrow cells following short-term exposure to drugs distinguish cell-cycle dependent drugs (*e.g.*, 5-fluorouracil) from cell-cycle independent agents [*e.g.*, melphalan, nitrogen mustard (9)]. Our studies clearly indicated that NSC 239851 has no such cell cycle dependence. This compound, therefore, may be administered daily in small doses or intermittently in high doses.

The cytotoxicity of this compound to murine CFU-C was significantly influenced by the change in the exposure temperature, thereby suggesting that the transport of this compound is energy-dependent active transport. This finding is in contrast to that of its inorganic congener, *cis*-diamminedichloroplatinum, in which relatively little temperature dependence of cytotoxicity was demonstrated (11). Changes in the ligands may be responsible for the difference in the temperature dependence of the 2 compounds. Goldenberg *et al.* (6) presented evidence that drug resistance of murine fibroblast cell lines to nitrogen mustard may partly be due to decreased membrane transport of the agent. Studies of temperature dependence of cytotoxicity of agents may prove useful in the eludication of the structure-efficacy relationship of chemotherapeutic agents.

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