Effects of cis-Diamminedichloroplatinum (NSC 119875) on Murine and Human Hemopoietic Precursor Cells

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SUMMARY

The effects of cis-diamminedichloroplatinum (NSC 119875) on murine and human hemopoietic precursor cells were studied in culture. When murine cells from normal and actively regenerating marrows were exposed to cis-diamminedichloroplatinum in culture, washed free of the agent, and assayed for the surviving hemopoietic precursor cells, similar sensitivity curves were obtained. This result indicates the absence of cell-cycle dependency of cis-diamminedichloroplatinum. When marrow cells were exposed to cis-diamminedichloroplatinum in culture at various temperatures, only minimal reduction of cytotoxicity was noted at 4°C. This observation, unlike that from the experiment with nitrogen mustard, suggests that the transport of this agent is by passive diffusion. Finally, when the sensitivity of human hemopoietic precursor cells to this agent was assessed using similar conditions and compared to that of murine hemopoietic precursor cells, a significant species difference was observed.

INTRODUCTION

Since the discovery of antiproliferative properties of platinum compounds by Rosenberg et al. in 1965 (17), many platinum congeners have been developed and studied for their potential effectiveness in the treatment of neoplasms. Some of them have been found to possess significant antitumor activity against murine tumors (5) and are considered to represent a new, unique class of chemotherapeutic agents. One of the original compounds, cis-diamminedichloroplatinum (NSC 119875), has been evaluated for human toxicity and is now available for clinical evaluation. Preliminary data suggest that the efficacy of the agent does not depend on cell cycle differences, its mode of transport is via diffusion, and that it is less toxic to the human hemopoietic precursor cells than to the corresponding cells of mice.

MATERIALS AND METHODS

cis-Diamminedichloroplatinum (II) (NSC 119875). cis-Diamminedichloroplatinum was synthesized using the method described by Kauffman and Cowan (11). Authenticity was confirmed by infrared spectroscopy, using a Perkin Elmer Model 467 infrared spectrometer, and by elemental analyses (Galbraith Laboratories, Knoxville, Tenn.). It was dissolved in 0.9% NaCl solution and then added to culture tubes containing marrow cell suspension.

Culture Assay for Murine Hemopoietic Precursor Cells. Bone marrow cells were obtained from femurs of SCH:ARS(ICR)F mice (Sprague Dawley, Madison, Wis.) as described previously (19). Marrow cells capable of forming colonies in culture were assayed by a modification of the technique described by Worton et al. (21). α medium (x8) (Flow Laboratories, Rockville, Md.) was used instead of Connaught Medical Research Laboratory Medium 1066 (Willowdale, Ontario, Canada), and bovine serum albumin was omitted. Marrow cells in single cell suspension were immobilized in 0.8% methylcellulose (Dow Chemical Co., Midland, Mich.), 20% fetal bovine serum (Flow Laboratories, Rockville, Md.), 20% conditioned medium made from murine L-cell monolayers, and α medium. Incubation was carried out at 37°C in a humidified 5% CO₂ in air atmosphere for 7 days, and the enumeration of colonies was done using an inverted microscope. Cells thus plated yielded 100 to 150 colonies, as reported by Worton et al. (21).

Cells from Regenerating Bone Marrow. Mice with regenerating bone marrow were prepared by the i.v. injection of 1 × 10⁶ nucleated marrow cells into lethally radiated isologous animals. They had received 850 rads of total body radiation using 250 kVp G. E. Maxitron unit at 30 ma and a quarter cupper filtration. The treatment distance was 50 cm and no cone was used. The machine calibration was checked immediately prior to the radiation procedure using Victoreen condenser R meter 570, probe 651. The femoral marrow of these mice was harvested 6 days later.

Assay for Human CFU-C. Bone marrow cells were...
obtained from patients by iliac marrow aspiration during the course of hematological assessment. Only patients whose marrow examinations were normal, other than absence of iron, and who had not received chemotherapy, were selected for study. The technique by Iscove et al. (10) was used with modifications consisting of the substitution of α medium for McCoy's 5A and omission of bovine serum albumin. Conditioned medium was obtained from fluid overlaying normal peripheral leukocytes immobilized in agar at the concentration of 10⁴/ml and incubated for 7 days. Cells were cultured in the presence of 0.8% methylcellulose, 20% conditioned medium, and 20% fetal bovine serum in the atmosphere described for murine CFU-C. After 10 to 14 days of incubation, colonies were counted using the criteria reported previously (12). Marrow cells thus plated yielded 14 to 45 colonies/10⁴ cells, corresponding to the plating efficiency reported by Iscove et al. (9).

**EXPOSURE TO DRUGS IN CULTURE.** Both murine and human bone marrow cells at a concentration of 10⁴ nucleated cells/ml were incubated with varying concentrations of cis-diamminedichloroplatinum for 1 hr at 37° using 16-ml Falcon tissue culture plastic tubes. Following drug exposure, cells were washed twice with cold α medium. Based on the preincubation cell counts, murine cells in final 1 x 10⁴ and human cells in 2 x 10⁴ cells/dish were plated in quadruplicate. The mean and the S.E.'s of the mean of the quadruplicate were calculated using a Wang 600 series programmable calculator, and the results were expressed as percentage survivals of the drug-treated groups with the control of 100%. The relevance of this test system in the evaluation of chemotherapeutic agents has been presented in the previous publications (13, 14).

**RESULTS**

**Cell-Cycle Dependency.** Marrow cells that are active in cell proliferation have been shown to be more sensitive than those from steady-state marrow both in vivo (3) and in culture (14) to such chemotherapeutic agents as vinblastine and 5-fluorouracil. Cells from both normal and regenerating marrows were exposed to increasing concentrations of cis-diamminedichloroplatinum in culture (see "Materials and Methods") and were assayed at a concentration of 1 x 10⁴ nucleated cells/ml for surviving CFU-C. The results are expressed as percentage survivals of the drug-treated groups with the control of 100%. The relevance of this test system in the evaluation of chemotherapeutic agents has been presented in the previous publications (13, 14).

**Effect of Temperature.** Membrane transport of the cis-diamminedichloroplatinum was studied indirectly using gradients of temperature. It has been shown that active transport (7) and murine CFU-C cytotoxicity (16) of HN2 are temperature dependent. In this study, murine marrow cells were exposed to cis-diamminedichloroplatinum in concentrations of 20 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 approximately 3% of control at 37° (Chart 1). The results are described using open circles in Chart 2. For comparison, a simultaneous temperature survival experiment was carried out using HN2 at a concentration of 0.5 μg/ml. The result is presented in Chart 2 using open squares and is in agreement with the previously reported studies.
Presented. Sensitivity of murine CFU-C to this agent described in the surviving fraction of CFU-C. Results from 4 different subjects are platinum in culture. Human marrow cells were exposed to concentrations of cis-diamminedichloroplatinum at 10 µg/ml, and the results are shown in Chart 2 using closed circles. Even with this lower concentration of the agent, about 50% cytotoxic activity was noted at 4°C.

Effect of Human CFU-C. The sensitivity of human hemopoietic precursor cells to cis-diamminedichloroplatinum was studied in culture using methods similar to those for murine CFU-C. Marrow cells, exposed to varying drug concentrations and washed, were assayed in 2 x 10⁴ cells/ml concentration for the surviving CFU-C. The results are expressed as percentage survival of CFU-C, and the mean and S.E.’s of the mean of 4 experiments are presented in Chart 3. When compared to the sensitivity of murine CFU-C, taken from Chart 1 and shown with a dashed line, the D₀ for human CFU-C (16 µg/ml) was 3 times as high as that of murine CFU-C (5.3 µg/ml).

DISCUSSION

Platinum antitumor compounds appear to constitute a new, unique class of cancer chemotherapeutic agents. cis-Diamminedichloroplatinum is currently being tested for its clinical effectiveness in patients with various cancers. The purpose of studies reported in this paper is to seek the information useful for rational administration of this compound. Bruce et al. (3) presented data showing that actively regenerating hemopoietic stem cells are more sensitive than those from the steady-state marrows to such chemotherapeutic agents as vinblastine or 5-fluorouracil and provided a rationale for the current high-dose intermittent chemotherapy (2). The fraction of marrow stem cells in cell cycle is larger in the regenerating marrow than in the 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. Our studies clearly indicate that cis-diamminedichloroplatinum has no such cell-cycle dependency and agree with the data reported by Drewinko et al. (4) that cytotoxicity of this agent is similar in all stages of the cell cycle.

Platinum compounds are effective both as a single agent or in combination with other classes of chemotherapeutic agents (8, 20). cis-Diamminedichloroplatinum possesses synergism with HN2 despite their similarity in postulated mode of action. The results depicted in Chart 2 suggest that the membrane transport of this agent is via passive diffusion, in contrast to HN2, which is dependent on temperature-sensitive active transport. Although indirect in the assessment of membrane transport, this study of cis-diamminedichloroplatinum agrees with the data by Gale et al. with a [3H]dipyridine-labeled organic analog of cis-diamminedichloroplatinum (6). It is possible that the mechanisms underlying this synergism is the difference in the membrane transport of these agents.

While dose survival responses of both murine and human CFU-C were linear on semilogarithmic scale, human cells were less sensitive to cis-diamminedichloroplatinum than are murine CFU-C, a relation similar to that with HN2 as reported previously (15). Species differences in the intrinsic sensitivities to platinum compounds such as described will be useful in extrapolating data from mouse to man for individual platinum compounds.

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