The Effect of cis-Diamminedichloroplatinum(II) on Cultured Human Lymphoma Cells and Its Therapeutic Implications

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SUMMARY

Asynchronous human lymphoma cells treated for 1 hr with increasing concentrations of cis-diamminedichloroplatinum(II) (DDP) revealed a marked decrease in survival as estimated by the colony-forming technique. When the treatment was extended for 8 hr, a killing effect (more than 3 log decades) was observed for a concentration of 5 µg/ml similar to that obtained with 50 µg/ml incubated with the cells for 1 hr. This finding suggests that better antitumor results with less toxic effects may be obtained clinically by prolonged infusion of low doses of DDP.

Synchronized lymphoma cells showed no significant degree of cell cycle stage sensitivity to DDP. The drug kills cells with similar efficiency in all stages of the cell cycle. No killing effect was elicited after incubation of the cells with spironolactone, a compound said to protect the kidneys from the toxic effect of heavy metals. However, simultaneous incubation of spironolactone and DDP did not prevent the lethal action of the second drug. If spironolactone is proven an inhibitor of DDP nephrotoxicity, it will become a valuable addition to the treatment of human neoplasias with DDP.

INTRODUCTION

Platinum compounds are a new class of antineoplastic agents. The most active inhibitors of cell proliferation are neutral complexes of cis isomers of platinum(II) and platinum(IV) (22). The original observations of Rosenberg, et al. (20, 21) on the lethal effects of platinum complexes on Escherichia coli lead to an evaluation of such drugs in the control of neoplastic proliferation. Platinum inhibits the growth of a variety of experimental tumors (16, 19, 25, 26) and also acts as an immunosuppressive agent (2, 10, 15). Recently, Phase I clinical studies have been initiated to evaluate the possible use of platinum complexes in chemotherapeutic protocols (13). Preliminary results indicate that these drugs may provide effective therapy in certain solid tumors. However, toxic effects, especially nephrotoxicity, delayed bone marrow suppression, and audiological impairment, may severely curtail their use (23).

Although a considerable amount of information is available concerning the influence of platinum compounds on biomolecular mechanisms and on their pharmacological properties (1, 4, 10, 12, 14, 18, 27), very little is known on the effects of these drugs at the cellular level. The present report analyzes the lethal effects of 1 such platinum complex, DDP, which actually is used clinically, on a long-term culture of human lymphoma cells by the colony-forming technique.

In addition, we studied the effects of spironolactone on the same lymphoma cell line used singly or in combination with DDP. It has been reported that spironolactone protects the kidneys from the toxic effects of heavy metal poisoning (11). The possibility exists that nephrotoxicity will be decreased by using spironolactone in conjunction with DDP, if the former drug does not interfere with the killing activity of the latter drug on neoplastic cells.

MATERIALS AND METHODS

A long-term culture of human lymphoma cells was utilized. Culture conditions, harvesting methods, and kinetic parameters have been previously reported (8, 9). Synchronization was carried out as described before (5, 6). DDP manufactured by Ben Venue Laboratories, Inc. (Bedford, Ohio), was obtained from the Cancer Chemotherapy Branch, National Cancer Institute, as a dry powder. The drug was dissolved in 0.9% NaCl solution and appropriate concentrations were made with fresh medium. The pH of the solutions ranged from 7.0 to 7.3. In all experiments the cells were exposed to DDP at 37° in a 5% CO2 humid atmosphere. After exposure, the drug was decanted and the cells were washed twice with fresh medium. The cells were harvested and counted with the aid of an electronic particle counter, and appropriate cell suspensions were seeded into 60-mm Petri dishes. The cells were incubated at 37° for 3 weeks and the medium was discarded. The colonies were washed with 0.9% NaCl solution, stained with crystal violet, and scored under a stereomicroscope. Colonies of 50 or more cells were considered to originate from viable cells. Percentage of survival was calculated in reference to controls (cells not exposed to drug). All experiments were done at least in duplicate with triplicate samples per dose or time point. Means and standard errors were calculated with the aid of a Sigma 5 XDS computer. To obtain dose-


2 The abbreviation used is: DDP, cis-diamminedichloroplatinum(II).
response survival curves, asynchronous cells in logarithmic growth were exposed to increasing concentrations of DDP for 1 hr. In other experiments the survival of asynchronous cells as a function of duration of treatment was investigated by exposing replicate cultures to a single concentration of 5 μg of DDP per ml for up to 8 hr. Controls were obtained at each time point.

Synchronized cells were incubated with a single dose of either 5 or 10 μg/ml for 1 hr at regular intervals throughout the cell cycle. In other experiments dose-response survival curves were determined at selected points of the cell cycle. Synchronization was monitored by pulse labeling replicate cultures for 30 min with 1 μCi of thymidine-3H per ml (specific activity, 3.0 Ci/m mole) at regular intervals. The cells were then washed twice with medium and harvested, and slide preparations were made with a cytocentrifuge (Shandon Scientific Co., Inc., Sewickley, Pa.) (7). Radiographs were made by the liquid emulsion technique utilizing a 50% solution of Ilford K-5. After 1 week exposure, the slides were developed in Kodak D19 and fixed in acid fix, and the cells were scored as labeled (5 or more grains overlaying the nucleus) or unlabeled under a phase microscope. In all experiments a synchrony of 90% of cells in S phase, 70 to 80% in G2, and 70% in G1 was obtained.

T4 cells were also treated with increasing concentrations of spironolactone (Aldactone; Searle and Co., San Juan, Puerto Rico). The 25-mg tablets were pulverized in a mortar and diluted in 25 ml of distilled water obtaining a clear solution which was filtered through a 0.20-μm Nalgene filter (Sybron Corp., Rochester, N.Y.). Drug concentrations were prepared as usual and the cells were treated for 1 hr. Survival was estimated in routine fashion. In other experiments, the cells were exposed to a combination treatment of increasing concentrations of DDP admixed with 50 μg of spironolactone per ml. After a 1-hr incubation, survival was determined as usual. Controls of increasing concentrations of DDP and spironolactone were also run in parallel.

RESULTS

Asynchronous T4 cells incubated for 1 hr with increasing concentrations of DDP exhibited an exponential decrease in survival as a function of the concentration of the drug with a mean lethal dose (D0) of 5.5 μg/ml (1 hr). There was no shoulder region in the survival curve (Chart 1).

Asynchronous T4 cells exposed to a single dose of DDP (5 μg/ml) showed an exponential decrement in survival as a function of duration of treatment (Chart 2). After 8 hr incubation with DDP the survival decreased by more than 3 log decades.

Synchronized cells incubated with either 5 or 10 μg of DDP per ml for 1 hr at various points of the cell cycle demonstrated some fluctuation in survival depending on the stage of the cycle. While mid-S-phase and late S-phase cells appeared relatively more resistant, G2 and especially G1 cells showed increased sensitivity to the drug. This effect was more evident with the 10-μg/ml concentration, where the difference in survival between the more resistant and the more sensitive cells was about 5-fold, while such difference was only 2-fold with the 5-μg/ml concentration. Hence, DDP does not appear to be a particularly stage sensitive drug, at least with clinically relevant concentrations. This fact is further illustrated in Chart 4 where cell survival, as a function of the concentration of DDP incubated for 1 hr with synchronized cells at selected points of the cell cycle, is shown. The slopes of the survival curves are very similar for G2, G1, and mid-S-phase cells (D0 = 5, 4, and 6 μg/ml, respectively) indicating that DDP kills cells with equal efficiency regardless of the stage of the cell cycle.

T4 cells incubated for 1 hr with increasing concentrations of spironolactone (5 to 100 μg/ml) failed to show any decrease in survival. Cells incubated for 1 hr with increasing concentrations of DDP admixed with 50 μg of spironolactone per ml revealed an almost identical decrease in survival to that observed when DDP was administered alone.

DISCUSSION

Platinum compounds have been shown to be efficacious in the control of neoplastic proliferation in experimental tumors and to have some effect in human neoplasia. Patients treated with a single injection of DDP show a brief initial peak of drug in serum with a long drawn-out serum clearance curve that may last several hr leading to some cumulative effects (Ref. 4; C. Steuart, personal communica-
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The mode of action of DDP is still in doubt. Preliminary studies have suggested that DDP may exert its lethal effect by cross-linking DNA (14, 18).

Our investigations focused on the effects of DDP at the cellular level, as an in vitro model of the lethal activity of the drug once it reaches the target cells in vivo. Pharmacological factors cannot be evaluated with this system, but the data provide information on the time and dose parameters necessary to affect cellular viability, which may guide the development of rational clinical chemotherapeutic protocols. In addition, the stage of the cell cycle where the drug exerts its preferential activity, if any, can thus be determined.

Our data indicate that DDP is an effective drug in reducing the survival of cultured human lymphoma cells with a $D_0$ of 5.5 $\mu$g/ml. Fifty $\mu$g/ml can decrease the survivors by more than 3 log decades. However, in the clinical situation, such plasma concentrations may only be reached with doses of 100 to 200 mg of DDP per sq m, which may cause considerable toxicity (4, 23). At the usual concentrations achieved in the serum of patients (2 to 5 $\mu$g/ml) (C. Steuart, personal communication) only a 50 to 75% reduction in survival was observed when the lymphoma cells were treated for 1 hr. However, since DDP has a low clearance from plasma and, in fact, may accumulate in extracellular fluids (4), a much greater killing effect may be achieved in vivo with such low concentrations. This can be seen in Chart 2 which mimics such a clinical situation by exposing the cultured cells to a continuous incubation with 5 $\mu$g of DDP per ml. After only 8 hr of continuous incubation, a killing effect (>3 log decades) is observed, similar to that obtained for cells exposed to 50 $\mu$g/ml for only 1 hr. The concentrations times time factor is lower for the former modality than for the higher dose short-term treatment. This fact suggests the need to investigate the efficacy of giving low doses of DDP in continuous infusion for extended periods of time. Usually, the use of prolonged infusion, as a mode of administering a drug, is dictated by cell kinetic properties of the agents, particularly its S-phase sensitivity (3, 24). In the particular case of DDP, this modality would be independent of stage sensitivity but only related to the possibility of obtaining greater antitumor effects with less clinical toxicity. Even though a single bolus injection of DDP may produce some accumulation of drug, the dose necessary to achieve an effective concentration in plasma is well beyond the nephrotoxic limit of 1.95 mg/kg (4) and most of the drug is rapidly excreted by the kidneys. A prolonged i.v. infusion of low doses of DDP should permit a steady plasma concentration under controlled conditions.

Synchronized T, cells exposed to a single dose of 5 or 10
µg of DDP per ml for 1 hr at 2-hr intervals throughout the cell cycle revealed a minor degree of stage sensitivity (Chart 3). This was more accentuated with the 10-µg/ml than with 5-µg/ml concentration. S-phase cells appeared relatively more resistant than G1 cells while the sensitivity of G2 cells was intermediate. Chart 4, depicting the dose-response survival curves of synchronized mid-S, G2, and G1 cells, readily demonstrates that there are no significant differential sensitivities along the cell cycle to DDP (D0 = 4 µg/ml, G1; 5 µg/ml, G2; and 6 µg/ml, S). These data clearly demonstrate that DDP cannot be considered a phase-sensitive drug.

One of the most critical untoward effects of DDP is its nephrotoxicity. The changes observed in the kidney are those common to heavy metal poisoning, especially mercury (17). Recently, Haddow and Lester (11) reported a protective effect of spironolactone in mercury poisoning of rats. These authors considered that spironolactone prevents toxicity by complexing with the heavy metal and increasing its excretion via the biliary tract. If this property of spironolactone extended to all heavy metals, including DDP, it could be beneficial in chemotherapeutic regimens which use DDP, by preventing nephrotoxic effects, provided that spironolactone did not interfere with the lethal activity of DDP on the target neoplastic cells. We explored the latter possibility by incubating the lymphoma cells simultaneously with DDP and spironolactone. No interference of the killing effect of DDP was noted. It follows that, if spironolactone does indeed protect the kidneys from DDP toxicity, it may become a valuable adjunct in platinum complexes therapy since it does not affect the lethal activity of the drug on the tumor population.

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