Effects of cis-Dichlorodiammineplatinum(II) on Human Colon Carcinoma Cells in Vitro

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ABSTRACT

The lethal effects of cis-dichlorodiammineplatinum(II) were investigated on an established human colon carcinoma cell line. cis-Dichlorodiammineplatinum(II) was one of the most efficient antineoplastic agents tested thus far on this human colon carcinoma cell line. Survival of exponentially growing cells exposed to increasing concentrations of the drug (both in medium or in Hanks' balanced salt solution) was of the threshold exponential type $D_0 = 1.2 \mu g/ml$, 1 hr; $D_0 = 3.5 \mu g/ml$, 1 hr). Stationary-phase cells were extremely sensitive to the drug, and the survival curve demonstrated a simple exponential pattern $D_0 = 3.9 \mu g/ml$, 1 hr). Long-term exposure to low concentrations of cis-dichlorodiammineplatinum induced a high degree of killing, with only 0.5% of the cells surviving after incubation for 24 hr with 2 $\mu g/ml$. Cells were unable to recover from potentially lethal or sublethal damages induced by the drug.

INTRODUCTION

We have recently investigated the action of DDP on a human lymphoma cell line, and some relevant characteristics of this antitumor agent were elucidated. The survival curve of exponentially growing lymphoma cells was of the simple exponential type $D_0 = 5.5 \mu g/ml$, 1 hr), no significant cell cycle stage sensitivity was noted, the lymphoma cells were unable to recover from sublethal damage, and DDP was shown to display considerable synergistic effects with several other antitumor agents (7, 8). Thus, we concluded that DDP represented an excellent antitumor agent for this type of cancer.

To investigate the properties of DDP further, we conducted similar studies on an established human carcinoma embryonic antigen-producing colon carcinoma cell line. The objectives of the study were (a) to evaluate the lethal effects of DDP on this cell line and (b) to compare the antitumor effects of DDP on 2 histologically different human cell lines.

MATERIALS AND METHODS

Cell Line. Cells used in this investigation (designated LoVo) are from a carcinoembryonic antigen-producing colon adenocarcinoma cell line. Biological properties and growth kinetics characteristics have been published elsewhere (9, 10). Monolayer cultures are maintained in Ham's F-10 medium supplemented with 20% fetal calf serum, vitamins, glutamine, and antibiotics. Harvesting procedures include successive treatment by hyaluronidase (100 units/ml, 5 min at 37°) and 2.5% trypsin (5 min at 37°). Under these conditions, the doubling time is 36.3 hr, the mitotic index is 0.8%, the labeling index is 31%, the growth fraction is 90%, and the generation time is 29.3 hr (10).

Cell Survival Experiments. Stock cultures of LoVo cells were harvested and counted with the aid of an electronic particle counter (Model ZBI Coulter counter; Coulter Electronics, Inc., Hialeah, Fla.). Cell suspension aliquots were seeded into 60-mm Petri dishes (0.5 x 10^6 cells/plate). The cells were incubated at 37° in a 5% CO_2 atmosphere in air for about 48 hr to achieve exponential growth. The medium was discarded, and the cells were exposed to increasing concentrations of DDP for 1 hr at 37°. DDP was obtained from the Division of Cancer Treatment, National Cancer Institute, manufactured by Ben Venue Laboratories, Inc., Bedford, Ohio, and dissolved in distilled water immediately before each experiment; appropriate concentrations were made by dilution with fresh medium. After incubation for 1 hr, the drug was discarded, and the cells were washed twice with Hanks' balanced salt solution, harvested as a monodispersed suspension, and counted. Known aliquots were dispensed into 60-mm Petri dishes so that 50 to 100 colonies would appear after incubation for 20 days in a 5% CO_2 humidified atmosphere at 37°. The colonies were stained with 2% crystal violet in 95% ethanol. Viability was defined as the ability of single cells to give rise to a colony of greater than 50 cells. In each experiment, the plating efficiency of at least 6 control cultures was assessed simultaneously. Control cultures consisted of cells treated in exactly the same way as the test cells but without receiving drugs. The survival fractions for the different drug concentrations were normalized with respect to the individual controls for each experiment. In this series of experiments, the plating efficiency ranged from 20 to 55%. All experiments were repeated at least twice with triplicate samples for each drug concentration and exposure time.

To test possible drug inactivation by the serum proteins contained in the medium (5), experiments were also performed by exposing exponentially growing LoVo cells to DDP diluted in Hanks' balanced salt solution instead of fresh medium; after exposure for 1 hr to DDP in Hanks' balanced salt solution, cells were washed, harvested, and plated in growth medium for colony formation.

For experiments involving cells in stationary phase, 5 x 10^6 LoVo cells were seeded in 60-mm Petri dishes and...
allowed to grow for 8 days, at which time the cell population growth rate reached a plateau; there were no mitotic cells, and the labeling index was 1% (30). Survival assays were conducted as described above.

To investigate survival as a function of length of incubation, exponentially growing cells were incubated with a constant concentration of drug for various intervals before harvesting for colony formation, and survival was assessed as usual.

Recovery. To investigate recovery from sublethal damage induced by DDP, cells were exposed to fractionated doses of the integral dose. Integral dose is defined as the product of the concentration times the time of incubation (C x t). Thus, asynchronous exponentially growing cells received the total integral dose in 2 equal separate exposures (15 µg/ml for 15 min each) spaced by increasing periods of time, and their survival was compared to that resulting from cells receiving the total integral dose at one time (30 µg/ml for 15 min). Controls obtained at regular intervals consisted of cells receiving (a) one-half of the total integral dose (15 µg/ml for 15 min), (b) the total integral dose (30 µg/ml for 15 min), and (c) the total integral dose, where the time parameter only was changed (15 µg for 30 min).

To test recovery from potentially lethal damage, we used the method described by Little (22). Cells treated with increasing concentrations of DDP were washed and reincubated at 37° with spent medium for 8 or 24 hr before harvesting and processing for colony formation. Spent medium was the supernatant from 2-week-old stock cultures.

RESULTS

The survival curve of exponentially growing LoVo cells was of the threshold exponential type (Chart 1). The curve shows a very slight shoulder with insignificant cell killing [Dq = 1.2 ± 0.1 µg/ml, 1 hr], after which cell survival decreases exponentially as a function of increasing concentrations of DDP (Do = 3.7 ± 0.35 µg/ml, 1 hr). In contrast, the survival curve of LoVo cells in stationary phase is of the simple exponential type, i.e., without a shoulder region, although the slope (D0 = 3.9 ± 0.42 µg/ml, 1 hr) is similar to that of exponentially growing cells.

The pattern of the survival curve elicited by DDP diluted in Hanks’ balanced salt solution was similar to that observed with DDP diluted in fresh medium, demonstrating no significant inactivation of DDP as a result of binding to serum proteins for the duration of the incubation (1 hr).

Exponentially growing LoVo cells exposed to single concentrations of DDP (1 and 2 µg/ml) for various periods of time showed an exponential decrement in survival as a function of increasing treatment duration for periods of up to 24 hr. At a concentration of 2 µg/ml, survival decreased by greater than 99% after exposure for 24 hr (Chart 2). In these experiments, the drug-containing supernatants were used to treat previously untreated LoVo cells for 1 hr at 37°. The survival of these cells was not significantly different from that observed for cells treated for 1 hr with freshly prepared drug.

In separate experiments, the survival curves of exponentially growing LoVo cells exposed to increasing concentrations of DDP for various durations of incubation (1, 3, 12, and 24 hr) were established. From each survival curve, the concentration of DDP leading to a given biological effect (i.e., 50% killing) was estimated graphically, and the corresponding exposure dose (C x t) was computed. Table 1 demonstrates that killing efficiency is independent of changes of the concentration or time parameters; the same lethal activity (50%) was obtained for a concentration of 0.2 µg/ml for 24 hr as that observed for a concentration of 3.3 µg/ml for 1 hr.

Survival of cells exposed to fractionated treatment was
Relationship between biological effect and exposure dose \( (C \times t) \)

Exponentially growing LoVo cells were exposed for various durations of incubation to increasing concentrations of DDP (0.5, 1, 2.5, and 10 \( \mu g/ml \)). Concentrations corresponding to 50 and 10% killing were estimated from the corresponding survival curves.

<table>
<thead>
<tr>
<th>Duration of incubation (hr)</th>
<th>Concentration of DDP corresponding to 50% killing (( \mu g/ml ))</th>
<th>Exposure dose ( (C \times t) ) corresponding to 50% killing (( \mu g/ml ))</th>
<th>Concentration of DDP corresponding to 90% killing (( \mu g/ml ))</th>
<th>Exposure dose ( (C \times t) ) corresponding to 90% killing (( \mu g/ml ), hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.3</td>
<td>3.3</td>
<td>10.1</td>
<td>10.1</td>
</tr>
<tr>
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<td>1.4</td>
<td>4.2</td>
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<tr>
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essentially identical to that for cells treated with the integral dose in a single exposure; there was no significant increase in cell survival during the time intervals allowed for recovery between 2 fractionated doses, indicating that exponentially growing LoVo cells were unable to recover from sublethal damage induced by DDP (Chart 3).

Survival in exponential and stationary phase of DDP-treated LoVo cells (harvested 8 and 24 hr after incubation in spent medium) was not improved in comparison to that of cells harvested immediately after drug exposure, denoting absence of repair of “potentially lethal damage” (Chart 4).

DISCUSSION

Early clinical trials with DDP have shown that although effective control could be obtained in certain solid tumors, toxic effects, especially nephrotoxicity, were a limiting factor in extensive clinical application (4, 14, 17, 21, 26). Newly devised means of improving nephrotoxicity have recently renewed interest in this drug (1, 16, 23), and clinical trials using mild to high doses of DDP alone or in combination have produced encouraging results in a wide range of solid tumors (12, 27–29). Previous trials with low doses of DDP as a single agent in human colon carcinoma were disappointing (14, 19). However, more recent combination therapy with 5-fluorouracil and DDP has led to more encouraging results (13), and Corbett et al. (3) have shown that DDP was mildly to highly efficient on 4 experimental transplanted colon carcinoma tumors (3). These diverse results raise the question of the adequacy of initial clinical trials.

In our studies, the killing activity of DDP on cultured human colon cells was among the most powerful of that of all drugs studied thus far on these cells. The efficacy was much greater than that previously observed by us for cultured human lymphoma cells (7) and was similar to the efficiency reported for HeLa cells (24). The pattern of the dose-response survival curve on exponential growing cells was of the threshold exponential type characterized by initial negligible killing, followed by an exponential decrease in survival as the drug concentration was increased. The shoulder region had not been observed for human lymphoma cells but was noted by Roberts and Pascoe (24) for HeLa cells treated with this agent. Threshold exponential survival responses for LoVo cells have thus far been noted only following exposure to ionizing radiations (11) and radiomimetic agents (6). The biological action of DDP on neoplastic cells shows numerous similarities to the action of alkylating agents such as inhibition of DNA synthesis (15, 18) and cross-linking ability of complementary DNA strands (24). Also, the activity spectrum of DDP against experimental tumors is similar to that of alkylating agents, and Connors (2) observed cross-resistance against some alkylating agents and platinum derivatives in experimental tumors. These data suggest that the mechanism of action of DDP on exponentially growing LoVo cells is possibly related to that of radiomimetic agents and that the shape of the survival curve reflects the capacity of LoVo cells to absorb sublethal damage. This capacity is probably related to metabolic changes coinciding with changes in proliferative status inasmuch as the shoulder region was absent for LoVo cells in stationary-phase growth. These results indicate that patterns of survival curves elicited after treatment with antitumor agents are a function not only of the drug but also of the species of cells used in the investigation and of their proliferative status.

LoVo cells (in a fashion similar to that of human lymphoma cells) were unable to recover from sublethal and
were washed and harvested either immediately (C) or after incubation in conditioned medium for 8 hr (A) or 24 hr (O). Bars, SE.

The authors express their gratitude to Catherine Green for her excellent technical assistance.

REFERENCES

7. Drewinko, B., and Gottlieb, J. A. Action of cis-dichlorodiammineplatinu

empotential lethal damage both in exponential and stationary phases of growth. These data indicate that damage inflicted by DDP is fixed and cannot be modified by cellular activity.

The same biological effectiveness, in terms of percentage of survival of LoVo cells, could be obtained by simple permutation of the equation \( C \times t \). Thus, similar survivals were obtained for LoVo cells treated with low concentrations (2 \( \mu \)g/ml) for prolonged intervals as for cells treated for 1 hr with much higher concentrations of DDP. Hence, if LoVo cells accurately reflect the behavior of in vivo cells, greater lethal effects of DDP on colon tumors cannot be anticipated by clinical tactics using prolonged treatment (i.e., continuous i.v. infusion).

In conclusion, DDP is a very efficient agent against an established human colon carcinoma cell line, the cells of which were unable to repair potentially lethal and sublethal damage induced by the drug. Furthermore, DDP was as efficient on stationary-phase LoVo cells as on exponential cells, an especially interesting observation in view of the known low labeling index of human colon carcinoma cells in vivo (20, 25). These results suggest that DDP merits new clinical trials in the treatment of colon carcinoma, keeping in mind that the response observed for LoVo cells may be obtained only for patients whose tumors present the biological properties (i.e., morphological differentiation, ploidy, etc.) displayed by this particular cell line.

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REFERENCES

7. Drewinko, B., and Gottlieb, J. A. Action of cis-dichlorodiammineplatinu

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