Hyperthermic Potentiation of cis-Diaminedichloroplatinum(II) Cytotoxicity in Chinese Hamster Ovary Cells Resistant to the Drug

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ABSTRACT

The cytotoxic and pharmacological properties of hyperthermia and cis-diaminedichloroplatinum(II) (DDP) were studied in DDP-sensitive and DDP-resistant Chinese hamster ovary cells in vitro. Cytotoxicity was measured by cell survival using colony formation assay and cellular platinum levels were determined by atomic absorption spectrophotometry. Hyperthermia potentiated DDP cytotoxicity in both DDP-sensitive and DDP-resistant Chinese hamster ovary cells. Dose enhancement ratios increased from 1.4 to 6.5 over the temperature range of 39–43°C. Cellular accumulation of platinum at 37°C in the sensitive cells was 2.3- to 3.3-fold greater than that in the drug-resistant cells. Cellular accumulation of DDP was increased by factors of 1.5 and 2.2 at elevated temperature. DDP resistance did not confer cross-resistance to heat alone. The results suggest that hyperthermia could be used to circumvent DDP resistance.

INTRODUCTION

DDP is an antitumor agent which may work by blocking DNA replication by covalent binding to guanine base pairs and formation of interstrand and intrastrand cross-links (1–3). It shows remarkable activity against squamous cell carcinoma of the head and neck, ovarian carcinoma, and testicular tumors (4). Many human tumors show a marked early response to DDP-based chemotherapy regimens but rapidly demonstrate drug resistance following repeated administration. Emergence of drug-resistant cell populations renders further chemotherapy with the same agents ineffective, and combination therapy with non-cross-resistant drug regimens has met with limited success (5, 6). To overcome drug resistance, new and more effective agents are needed, or novel ways must be devised to use those drugs that are already available.

Hyperthermia has shown some promise as an adjunct to radiation therapy (7). It also has been shown to enhance the cytotoxic effects of several chemotherapeutic agents, including BCNU (8), doxorubicin (9), mitomycin (10), and DDP (10, 11). While hyperthermia augments the response of DDP-sensitive cells to DDP, the response of DDP-resistant cells to concurrent hyperthermia remains to be examined. In the present study, we report that hyperthermia potentiates DDP cytotoxicity in DDP-resistant CHO cells.

MATERIALS AND METHODS

Cell Cultures. All experiments were performed with Chinese hamster fibroblasts, HA-1 cells, and their DDP-resistant variants, HA-C4 and HA-C6. The cells were grown in Eagle's minimal essential medium (Gibco, Santa Clara, CA) supplemented with 15% fetal calf serum and gentamicin (25 µg/ml). Cell cultures were kept at 37°C in a humidified incubator with a mixture of 95% air and 5% CO2 and routinely checked for Mycoplasma contamination. All experiments were done with cells in exponential growth phase, prepared by plating 3 x 10^4 cells in 60-mm Petri dishes on day 0. Experiments were done on day 2, when the cell density reached 0.8 to 1.2 x 10^5 cells/cm².

DDP-resistant cell lines, HA-C4 and HA-C6, were developed by growing cells in progressively higher concentrations of DDP. The HA-C4 line was grown in a final DDP concentration of 0.13 µg/ml for 2 weeks prior to being returned to drug-free medium. HA-C6 cells were grown in a final DDP concentration of 0.3 µg/ml for 2 weeks prior to being returned to drug-free medium. Both drug-resistant lines were passaged in drug-free medium for at least 1 month before being used in experiments. They were maintained from that time on in drug-free medium and showed no loss of resistance during the 6 months that the experiments were performed. The lines were not replaced with frozen stock during the study period. Plating efficiencies for all cell lines were 85–95%, with doubling times of 14, 16, and 16 h for HA-1, HA-C4, and HA-C6, respectively.

Drug Treatment. A DDP stock solution was prepared by diluting freshly mixed DDP (Bristol-Myers, Syracuse, NY; kindly supplied by Dr. James H. Keller, Bristol Laboratories) to a concentration of 100 µg/ml in sterile water. Multiple 1.5-ml aliquots were stored in the dark at —20°C. Immediately prior to each experiment, a vial of DDP was thawed to room temperature and diluted to the appropriate concentration with complete medium. After 1 h exposure to DDP, the drug-containing medium was removed and the cells were washed once with PBS, trypsinized, counted with a Coulter Counter, and plated after appropriate dilutions had been made. The dishes were incubated at 37°C for 8–10 days, at which time surviving colonies were stained with crystal violet and those with 50 or more cells were counted with a dissecting microscope. Whenever possible, dishes containing 50–200 colonies were used for calculation of survival.

Heat and Radiation. Heating of cell monolayers was done in water baths at the appropriate temperatures in specially designed incubators. The pH of the medium was maintained at 7.2–7.4 by a regulated flow of 95% air and 5% CO2. The temperature of the water bath was controlled to within ±0.1°C. Fresh medium, with or without DDP, was placed over the cells immediately prior to heating. Cells were irradiated with a Westinghouse Quadronex X-ray machine at 250 kVp and 15 mA and a dose rate of 300 rads/min.

Cellular Platinum Determination. Cellular platinum levels were determined using a Perkin-Elmer Model 2800 flameless atomic absorption spectrophotometer. Cell monolayers were exposed to DDP in full medium and immediately washed twice with PBS. The cells were then trypsinized, pelleted, resuspended in cold PBS (4°C), and repelleted. Cells were then resuspended in 40 µl of PBS and a 20-µl sample was pipetted into a pyrocoated graphite tube of a Perkin-Elmer HGA 400 graphite furnace. The oven was programmed for the following steps: step 1, drying, 40 s at 110°C; step 2, charring, 45 s at 1500°C; step 3, cleaning, 15 s at 20°C; step 4, atomization, 5 s at 2500°C; step 5, cleaning, 3 s at 2650°C. The spectrophotometer was set to measure absorbance at 266.9 nm and deuterium background correction was used. All experiments were performed in triplicate.

RESULTS

Fig. 1 shows the effect of sequencing on the interaction of DDP (1.0 µg/ml) with heat (41°C). Exposure of cells at 41°C in the absence of DDP produces no cell killing. Hyperthermic

Received 4/11/86; revised 8/4/86; accepted 8/19/86.

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1 The work was supported in part by Grants CA-31397 and CA-09215 from the National Cancer Institute, Department of Health, Education and Welfare; the Louis R. Larue Foundation; and the Randy Lynn Baruch Research Foundation.

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3 The abbreviations used are: DDP, cis-diaminedichloroplatinum(II) (cisplatin); BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline; DER, drug enhancement ratio.
HYPERTHERMIA AND DDP RESISTANCE

Fig. 1. Effect of sequencing on the interaction between hyperthermia (41°C) and DDP. Cells were exposed to DDP, 1.0 µg/ml, for 1 h at time 0. Data points are at the midpoint of 1-h heating intervals.

potentiation of DDP was maximal when both modalities were given simultaneously. There was little or no augmentation of DDP effect when the cells were heated before or after drug exposure.

Survival of the three cell lines following DDP exposure at various temperatures is shown in Fig. 2. At the 10% survival level, HA-C4 and HA-C6 are 1.4 and 2.5 times more resistant to DDP, respectively, than the HA-1 cell line. All cell lines showed enhanced cell killing at elevated temperatures. Exposure of cells to 39°C or 41°C in the absence of DDP produces no cell killing. Exposure to 43°C for 1 h gives a survival fraction of 0.6. In the curves shown, survival has been corrected for cell killing due to heat alone at 43°C. At all temperatures and drug concentrations tested, cell killing by combined heat and DDP was greater than that predicted by additivity alone, as defined by Valeriote and Lin (12).

DERs were calculated as the concentration of DDP needed to give a 10% survival in the absence of heat, divided by the concentration needed to give a 10% survival in the presence of heat (Fig. 3). For example, a DER of 2.0 at 41°C means that only one-half as much drug is needed to give a survival of 10% when combined with 41°C hyperthermia, as is needed at 37°C. For calculations of DERs at 43°C, survival was corrected for cell killing by heat alone. DERs were similar for the drug-sensitive (HA-1) and the drug-resistant cell lines at 39°C and 41°C, while the DERs for the drug-resistant cell lines were higher than that for the HA-1 line at 43°C.

The effect of heat alone on cell survival is shown in Fig. 4. There was no apparent pattern of altered heat sensitivity with the acquisition of drug resistance. At low survival levels, HA-C6 appears to be slightly more heat sensitive than the HA-1 cell line, while HA-C4 may be slightly less heat sensitive.

Fig. 5 shows the accumulation of platinum in HA-1 cells and in the drug-resistant cell line, HA-C6. Platinum accumulation in the drug-resistant cells was markedly reduced compared to the drug-sensitive cells. Following 1 h exposure to DDP, 1.0, 3.0, and 5.0 µg/ml, at 37°C, HA-C6 cells accumulated only 44, 35, and 30% as much platinum, respectively, as did the HA-1 cells (as determined by integration of the areas under the curves).

Fig. 6 shows the cellular accumulation of platinum at 37°C and at 43°C. By integrating the areas under the curves, it was found that hyperthermia increased platinum accumulation by factors of 1.5 and 2.2 in the DDP-sensitive and -resistant cell lines, respectively.

DISCUSSION

Several investigators have shown that hyperthermia acts synergistically with DDP with in vitro tumor models (10, 11). Marmor (13) showed that hyperthermia and DDP act synergistically against murine tumors in vivo. In agreement with pre-
vious investigators, we found that hyperthermia and DDP act synergistically at 39–43°C in drug-sensitive HA-1 cells. The degree of hyperthermic enhancement of DDP cell killing in our cells is similar to that reported by Barlogie et al. (10) using human colon carcinoma cells. We found a similar degree of synergism between hyperthermia and DDP in DDP-resistant cells.

The temperatures and drug concentrations (14) used in the experiments reported here are within the ranges that can be achieved clinically. Temperatures of 39–41°C are achievable with whole body hyperthermia, while temperatures of 42–43°C can be achieved with localized hyperthermia (7). To date, preliminary studies in humans with whole body hyperthermia and chemotherapy have met with limited success (15, 16). Localized hyperthermia with temperatures of 42–43°C may be more effective. Our in vivo data would predict a greater DER at higher temperatures. Additionally, a greater therapeutic gain would be likely with localized hyperthermia because only those tissues in and around the tumor would be exposed to DDP at elevated temperatures.

Cisplatin resistance in the HA-C6 cell line is associated with decreased cellular accumulation of platinum as compared with the drug-sensitive parent cell line. HA-C6 cells are 2.5-fold more resistant to DDP than HA-1 cells, and platinum accumulation in the parent cell line was 2.3 to 3.3 times as great as in the resistant cell line. Decreased platinum accumulation may not fully explain drug resistance at 37°C. Other factors, including altered DNA repair, drug efflux, or drug metabolism may contribute to DDP resistance.

Hyperthermic potentiation of cisplatin toxicity is associated with increased cellular platinum accumulation. Hyperthermia (43°C) increases cellular accumulation of platinum by factors of 1.5–2, while the dose enhancement ratios at 43°C were between 5.5 and 6.5. Enhanced adduct formation with critical structures, decreased repair of damage, or increased sensitivity of target structures may also contribute to increased DDP cytotoxicity at elevated temperatures. Meyn et al. (17), for instance, have shown that hyperthermia increases DNA cross-linking by DDP. More detailed kinetic and biochemical studies must be performed to delineate precisely the mechanism of DDP resistance and hyperthermic potentiation of DDP cytotoxicity.

Corry et al. (18) reported previously that simultaneous heating and DDP exposure gave maximal potentiation of DDP. In their report, however, there was still considerable interaction between DDP and hyperthermia when they were separated by as much as 2 h. We found a much more pronounced decrease in hyperthermic potentiation of DDP if heating and drug exposure were not done simultaneously. Optimal sequencing will be very important if thermochemotherapy is to be effective.

Our CHO cells showed no significant changes in heat sensitivity with acquisition of DDP resistance. Da Silva et al. (19)

![Fig. 3. Dose enhancement ratios for HA-1, HA-C4, and HA-C6. Survival was corrected for killing due to heat alone at 43°C prior to calculation of the dose enhancement ratios.](image)

![Fig. 4. Survival curves for exposure to heat alone. Cells were trypsinized immediately after heating.](image)

![Fig. 5. Cellular platinum accumulation at 37°C in the parent cell line (HA-1) and the drug-resistant cell line (HA-C6).](image)
found no change in heat sensitivity in rat brain tumor cells selected for resistance to BCNU. Wallner and Li (20) reported no change in heat sensitivity associated with acquisition of doxorubicin resistance. We have found, however, that mitomycin C resistance in CHO cells is associated with increased heat sensitivity. Given the propensity for pleiotropic resistance among chemotherapeutic agents, it is encouraging that cross-resistance at the cellular level between heat and chemotherapeutic agents has not been demonstrated.

Synergism between hyperthermia and chemotherapeutic agents in drug-resistant cells has been demonstrated in cells selected for resistance to DDP and BCNU (19). In contrast, heat does not potentiate the action of doxorubicin in doxorubicin-resistant cells (20). If extrapolated to the clinical situation, these studies suggest that tumor cells which have developed resistance to doxorubicin may not respond any better to the drug in the presence of heat than they would at 37°C, while tumor cells which have developed resistance to BCNU or DDP might be treated effectively with the same drugs, in combination with hyperthermia.

ACKNOWLEDGMENTS

We thank John Phillips and Bruce Wilbur for their expert technical assistance.

REFERENCES

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