ABSTRACT

Hyperthermic enhancement of cis-diammine-1,1-cyclobutane dicarboxylate platinum(II) (carboplatin) cytotoxicity was studied in vitro in JM, a human acute lymphoblastic leukemia cell line. Corrected for direct heat toxicity, hyperthermia enhanced carboplatin killing at the clinically relevant temperatures of 40.5°C and 41.8°C. The thermal enhancement ratios at these temperatures were 1.89 and 3.32, respectively. Cell killing increased exponentially with increasing duration of combined treatment (41.8°C, carboplatin 30 μg/ml) for at least 3.5 h. Hyperthermic enhancement was maximal when heat was given during or immediately before carboplatin; enhancement was diminished when heat preceded carboplatin by more than an hour and was not apparent when heat followed drug treatment.

As carboplatin is associated with different clinical toxicity than is cis-diaminedichloroplatinum(II), carboplatin may represent an ideal drug in its class of anticancer agents to use in clinical whole body hyperthermia trials.

INTRODUCTION

The combination of hyperthermia and the chemotherapeutic agent cisplatin has generated considerable interest as an anticancer therapy. Cisplatin has shown significant clinical activity against many common types of cancer (1). In preclinical studies in vitro and in vivo hyperthermia has been found to enhance the tumoricidal effect of cisplatin (2–4). Clinically, local hyperthermia (i.e., limb perfusion) and cisplatin have been combined with reported therapeutic benefits (5).

Unfortunately, the combination of cisplatin and hyperthermia currently has limited clinical utility. For the vast majority of cancer patients local or regional hyperthermia may have only palliative benefits because of the sower of systemic metastases (6). Moreover, the combination of cisplatin and whole body hyperthermia has been associated with unacceptable clinical toxicity including serious renal injury not seen with other drugs using the same hyperthermia system (7). The etiology of this increased nephrotoxicity, while unknown, may be related to increased renal tissue levels of cisplatin as suggested from canine experiments (8). In addition, despite vigorous antiemetic prophylaxis, cisplatin frequently causes severe or intractable nausea and emesis (1, 9). Because of the risk of aspiration pneumonia these side effects are undesirable with any whole body hyperthermia system which does not require endotracheal intubation (6).

Carboplatin, a cisplatin analogue, is a promising agent to combine with whole body hyperthermia. Compared to cisplatin, carboplatin causes considerably less nausea and nephrotoxicity as well as less neurotoxicity and ototoxicity; yet carboplatin retains significant tumoricidal activity against a variety of cancers (10–13).

Little is known, however, about the effects of heat on carboplatin cytotoxicity. Our review of the literature reveals no reports regarding heat-carboplatin interactions and the nature of this interaction cannot be inferred. Heat could similarly affect carboplatin and cisplatin since these drugs may act on tumor cells by the same general molecular mechanisms (14). Alternatively, hyperthermia could interact differently with the two drugs since they differ both structurally (9, 14) and in their spectrums of toxicity (10–13). Ultimately, the dissimilar toxicities of carboplatin and cisplatin may reflect different events at the molecular level.

Because of the potential value of combining carboplatin and hyperthermia we investigated the effects of heat on carboplatin cytotoxicity. Drug dose, duration of treatment, and drug-hyperthermia sequencing were studied in vitro at temperatures which are clinically relevant for whole body hyperthermia.

MATERIALS AND METHODS

Cell Lines. Experiments were performed on JM, a human, T-cell, acute lymphoblastic leukemia cell line (15) provided by Dr. S. Z. Salahuddin at the National Cancer Institute. Initially cells were cloned in 0.30% Difco Bacto Agar (Difco Laboratories, Detroit, MI). A clone was selected, recloned in agar, and then grown for use in subsequent experiments. Periodically, old suspensions of this clone were replaced from frozen samples. Cells were maintained in exponential growth in RPMI 1640 (GIBCO Laboratories, Grand Island, NY) containing 20% fetal bovine serum (HyClone Laboratories Inc., Logan, UT), 100 units of penicillin and 100 μg streptomycin per ml (Sigma Chemical Company, St. Louis, MO). During experiments this solution was further supplemented with 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (“medium”) (Flow Laboratories, McLean, VA). Cells were grown in a humidified incubator containing 5% CO2 and 95% air at 37°C controlled to within 0.1°C.

Heat Treatments. Exponentially growing cells were centrifuged and resuspended in fresh medium. Pipeting several times gave at least a 99.9% single cell suspension. Aliquots of cells were then placed in 15 ml disposable, sealable plastic centrifuge tubes, left very lightly capped in the incubator for 10 min and then tightly sealed. This gave all cell samples a uniform, stable gas mixture and a stable pH of 7.4 ± 0.05 during treatments. Tubes were heated with continuous gentle shaking by suspension in water baths at 37.0, 40.5, or 41.8°C ± less than 0.05°C as determined by a mercury thermometer calibrated against a platinum resistance thermometer (Instrulab, Dayton, OH) accurate to 0.01°C. Temperatures within tubes reached both temperatures after 3 to 4 min.

Drug Treatments. Carboplatin (Drug Synthesis and Chemistry Branch, National Cancer Institute) was dissolved in medium immediately before each use, filter sterilized, and diluted to the necessary concentration. After drug treatments cells were washed with fresh drug-free medium.

Cloning of Cells after Treatments. Cells were cloned in top agar layers containing 0.30% Difco Bacto Agar and RPMI 1640 supplemented with antibiotics. Bottom agar layers contained 0.51% agar. Top and bottom layers, plated in 6-cm diameter disposable petri dishes, contained 20 and 10% fetal bovine serum, respectively. Cloning efficiency of untreated controls averaged 31%. Colonies were macroscopic by day 7 and colonies exceeding approximately 0.1 mm diameter (at least 50 cells) were counted after 14 to 18 days using 2-fold magnification.

All experiments were repeated at least twice.
RESULTS

Fig. 1 shows JM cell survival after simultaneous carboplatin and heat exposure. Cells were incubated for 1 h in 0–60 μg of carboplatin/ml at 37.0, 40.5, and 41.8°C. Data are corrected for direct heat killing which is 13.4% at 40.5°C and 58.6% at 41.8°C. At all three temperatures survival decreases exponentially without an apparent shoulder. The thermal enhancement ratio, calculated as the slope at an elevated temperature divided by the slope at 37°C, is 1.89 at 40.5°C and 3.32 at 41.8°C.

Fig. 2 shows the effects of drug-heat sequencing on carboplatin toxicity. Corrected for direct heat killing, an hour of hyperthermia (41.8°C) is most effective during or immediately before an hour-long carboplatin exposure (60 μg/ml). Hyperthermia ending more than 1 h before carboplatin causes less thermal enhancement. Little or no thermal enhancement occurs when heat follows drug exposure by as little as 17 min, the minimum time to wash carboplatin from cells and place them in the 41.8°C bath.

As is shown in Fig. 3, cell survival decreases exponentially with the duration of treatment during simultaneous heat (41.8°C) and carboplatin (30 μg/ml) exposure.

DISCUSSION

Hyperthermia markedly enhances carboplatin cytotoxicity as is shown in Fig. 1. These data are comparable to results seen in studies of heat-cisplatin toxicity in other exponentially growing (2–4, 16, 17) and plateau phase (18) cells. The results in Fig. 1 also show that temperatures which are safely achievable (6) using existing whole body hyperthermia techniques (up to 41.8°C) are high enough to markedly enhance carboplatin toxicity. As is also seen when heat and cisplatin interact (3, 4, 16, 17), the magnitude of carboplatin thermal enhancement increases with increasing temperature.

The sequence of heat and carboplatin exposure is another factor which strongly affects the magnitude of thermal enhancement (Fig. 2). Similar results are seen from existing in vitro studies of heat-cisplatin sequencing (16–18). In general, the present study and others ((16–18) support the conclusion that simultaneous drug and heat exposure is the most cytotoxic treatment sequence for carboplatin and cisplatin. In one study, however, heat (41°C) immediately before cisplatin causes much less thermal enhancement (16) than occurs when heat and cisplatin are given simultaneously. This dissimilarity may reflect differences in treatment temperature or varying rates of repair mechanisms in different cell lines.

The duration of treatment is one factor with little apparent impact on the degree of thermal enhancement when heat and carboplatin are given simultaneously. At both 37.0 and 41.8°C cell survival decreases exponentially as the length of exposure increases (Fig. 3). Even after correcting for direct heat toxicity, the ratio of killing at 41.8 to killing at 37.0°C remains constant for any treatment length up to at least 3.5 h. Consequently, at 41.8°C, relative enhancement of carboplatin toxicity remains independent of treatment duration. Parenthetically, after correcting for direct heat toxicity, killing by heat plus carboplatin is supra-additive.

Interestingly, the exponential decrease in survival shown in Fig. 3 is very different from the survival curve when JM cells are treated with heat and radiation. As we report elsewhere, when heat immediately precedes a constant dose of radiation, survival decreases exponentially with heating duration for the first 80 to 100 min of heating. Thereafter the survival curve changes sharply and additional heating causes little further augmentation of radiation killing. Heat may enhance drug and radiation toxicity by impeding repair of DNA damage (19). Consequently, the dissimilar effects of heat on carboplatin and radiation toxicity suggest that carboplatin and radiation cause different lethal lesions or that damage by these two agents may be repaired by different mechanisms in the JM cell line.

The data reported here support several clinically relevant conclusions. First, hyperthermia markedly enhances carboplatin cytotoxicity at temperatures which are safely achievable with whole body hyperthermia (Fig. 1). Thus, because of differences

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Fig. 1. Survival of JM in carboplatin at varying temperatures. Cells were simultaneously heated and exposed to varying concentrations of carboplatin for 60 min. Survival was corrected for direct heat killing and was calculated relative to that of untreated controls.

Fig. 2. Effect of treatment sequence on killing by heat and carboplatin. Cells were heated 60 min at 41.8°C at varying times before (negative time values), during (time 0), and after (positive time values) a 60-min carboplatin treatment at 60 μg/ml. Survival was calculated as a percentage of the survival of untreated controls and was corrected for direct heat killing. Points, midpoints of heat exposures; arrow, survival after carboplatin treatment alone at 37°C.

Fig. 3. Effect of treatment duration on survival. Cells were exposed to heat alone (41.8°C), to carboplatin alone (30 μg/ml), or to carboplatin and heat simultaneously and then plated at 30-min intervals. The data for combined heat and carboplatin are not corrected for direct heat killing.

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in toxicity between carboplatin and cisplatin, carboplatin may be an ideal drug in this class of antineoplastic agents for use in clinical whole body hyperthermia. Second, since the magnitude of thermal enhancement depends on temperature, changing treatment temperature is one way to modulate thermal enhancement of carboplatin cytotoxicity in clinical trials.

In contrast, changing the duration of combined carboplatin-heat exposure should not affect the magnitude of thermal enhancement. However, varying treatment duration may represent one way to control carboplatin's net cytotoxic effects in a predictable manner (Fig. 3).

Finally, the data presented here on heat-drug sequencing suggest that hyperthermia should be given during or perhaps immediately before carboplatin in future clinical trials.

REFERENCES

Hyperthermic Enhancement of cis-Diammine-1,1-cyclobutane Dicarboxylate Platinum(II) Cytotoxicity in Human Leukemia Cells in Vitro

Justin D. Cohen and H. Ian Robins