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Potentiation of platinum analogue cytotoxicity by hyperthermia*

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Summary. Carboplatin and iproplatin, two new analogues of cisplatin, appear to have comparable activity in the treatment of advanced ovarian cancer, but minimal nephro- and neurotoxicities. Hyperthermia can potentiate the cytoxicity of cisplatin in vitro and in vivo, but systemic treatment with the combination has proven unsafe in patients. To provide the rationale for an alternative approach, we evaluated the relative degree of additivity between hyperthermia and the three platinum analogues in vitro against a human ovarian adenocarcinoma cell line, UACC-66. All drug and heat treatments were simultaneous for 1 h. Platinum analogue concentrations covered a five-log range from 0.001 to 100 μg/ml and hyperthermia temperatures included 38.5°, 40°, 41.5°, and 43° C. A tumor clonogenic assay was used to quantitate heat-drug interactive effects against the UACC-66 cells, and statistical analysis was performed using the median-effect equation of Chou. When combined with heat, the in vitro concentrations of the three platinum analogues were between 5% and 25% of those required at 37° C to inhibit 50%–70% of the UACC-66 tumor colony-forming units. For each drug when combined with heat, a 3° C incremental increase in temperature (i.e., from 37° C to 40° C or from 40° C to 43° C) was associated with a ten-fold decrease in ID_{50} drug concentration. We conclude that the synergistic effects of both carboplatin and iproplatin with hyperthermia at all temperatures above 37° C provide a rationale for design of clinical trials in patients with ovarian cancer using these hyperthermia-drug combinations.

Introduction

Cisplatin has proven the most useful cytotoxic agent in the treatment of advanced ovarian cancer [6, 11, 23]. Recently, two new cisplatin analogues, carboplatin [13, 24] and iproplatin [5, 21] have also shown promising clinical activity in patients with ovarian cancer. While retaining a high degree of antitumor activity, the two new analogues have been associated with minimal nephrotoxicity and neurotoxicity, but myelotoxicity has proved dose-limiting [5, 7, 10, 13]. Thus, both of these agents may be associated with better therapeutic indices than cisplatin in the setting of ovarian cancer therapy.

Because of the significant synergism between cisplatin and hyperthermia in vitro and in vivo in preclinical models [1, 4, 18], clinical trials have combined regional hyperthermia with cisplatin in the management of advanced, recurrent cancers of various types. Unfortunately, regional hyperthermia, at least to the abdomen, may significantly enhance the nephrotoxicity of cisplatin. One report suggests that cisplatin should not be combined with hyperthermia because of the high risk of life-threatening renal and neurotoxicities [14]. Because of their relative lack of renal and neurological toxicities, carboplatin and iproplatin may prove less toxic and thus more efficacious when combined with hyperthermia in the treatment of patients with advanced cancers. Unfortunately, few data are available concerning the additive cytotoxic effects of hyperthermia and these two cisplatin analogues. We have used a human ovarian adenocarcinoma cell line to evaluate the relative activities of cisplatin, carboplatin, and iproplatin when combined with hyperthermia.

Material and methods

Cell line. A human ovarian cancer cell line (University of Arizona Cancer Center-66 or UACC-66) was utilized for this study. The UACC-66 cell line was isolated in 1983 by A. Leibovitz from a poorly differentiated ovarian adenocarcinoma metastatic to the abdominal wall of a 80-year-old woman. The cell line chromosome number is near triploid with structural abnormalities including t(1;13), t(12;13), iso 3q, iso 8q, iso 21q, $9p^+$, and $11q^-$. Neither HSR nor double minute chromosomes were observed. Just prior to use in the in vitro cloning assay experiments, frozen cells (−120° C) were thawed and grown as monolayer cultures in McCoy's 5A medium supplemented with 10% fetal calf serum and antibiotics. All incubations were carried out in a humidified atmosphere of 95% air and 5% CO_2. Under these conditions the cells have a doubling time of approximately 52 h. To assure reproducibility, all experiments used the same cell line passage number and cells in exponential growth phase.

Heat-drug treatment. Hyperthermic treatments were accomplished using a precision water bath which at 37° C

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had uniformity of ±0.03° C (Precision Scientific, Model 25, Chicago, Ill). Hyperthermia temperatures used in the experiments were 38.5°, 40°, 41.5°, and 43° C. Cisplatin and iproplatin were obtained from the Antitumor Biology Department, Research Division and Pharmacology Research and Development Division, Bristol Myers Co., Syracuse, NY. Carboplatin was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, Md. Stock solutions of all three drugs were made by dissolving 5 mg drug in 5 ml 0.85% NaCl solution. Appropriate dilutions were performed in complete medium just before the experiments. All drug and heat treatments used a 1-h exposure and were terminated by washing the cells twice with McCoy's 5A medium containing 2% fetal calf serum and maintained at 37° C.

Tumor cloning assays. After the heat-drug treatments, a double soft layer agar system of 0.3% on a 0.5% base layer was used, as described by Salmon et al. [20]. On each 35-mm petri dish, 15000 cells were plated and placed in a 5% CO_2 incubator at 37° C. About 2 weeks later, the plates were stained with a vital dye and then counted after 24 h using a Bausch and Lomb Omnicon FAS II image analysis system.

Data analysis. Heat-drug dose-effect relationships were analyzed by the median-effect equation derived by Chou [8]:

$$\text{fraction affected/fraction unaffected} = (\text{dose}/ID_{50})^m$$

where m is the slope of the median-effect plot (a measure of sigmoidicity).

The logarithmic form of the equation gives the basis for the median-effect plot:

log (dose) versus log (fraction affected/fraction unaffected)

To provide experimental data for the median-effect equation, we determined the inhibitory dose 50 (ID_{50}) concentration for each drug and heat used alone. The ratio of drug concentrations and heat at the appropriate levels was used to construct dose-survival curves for the combination of drugs with heat. The regression coefficient had to be at least 0.9 for the relationships to be valid. The slope and ID_{50} values were obtained from the linear regression equation.

The interaction of drugs with heat was determined by the combination index, which is defined by:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} + \frac{\alpha (D)_1 (D)_2}{(D_x)_1 (D_x)_2}$$

where D_x is the dose that is required to produce x% effect, and is dependent on the ID_{50} and slope of the single-agent dose-response curve. D is the combination dose which yields the same inhibition. It is dependent on the ID_{50} and slope of the combination dose-effect curve. For mutually exclusive agents, $\alpha = 0$. For mutually nonexclusive agents, $\alpha = 1$. CI = 1 indicates an additive interaction, whereas CI < 1 indicates a synergistic and CI > 1, an antagonistic interaction between the agents. Computer programs based on the above equations were used in the present studies for automated analyses of dose-effect data.

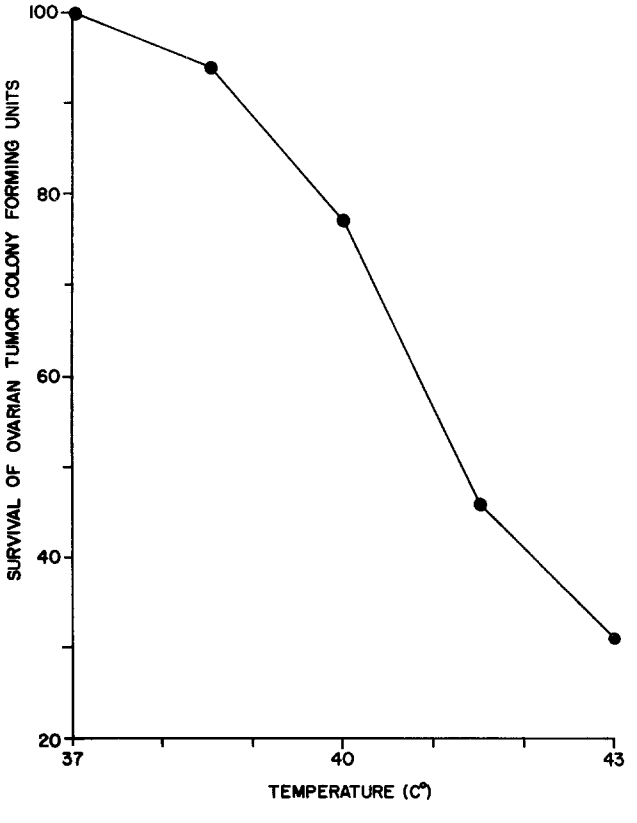

Fig. 1. Effect of heat on survival of tumor colony-forming units (TCFUs) of UACC-66 ovarian cancer cell line following a 1-h hyperthermic treatment. Each *point* represents the mean percentage survival of TCFUs

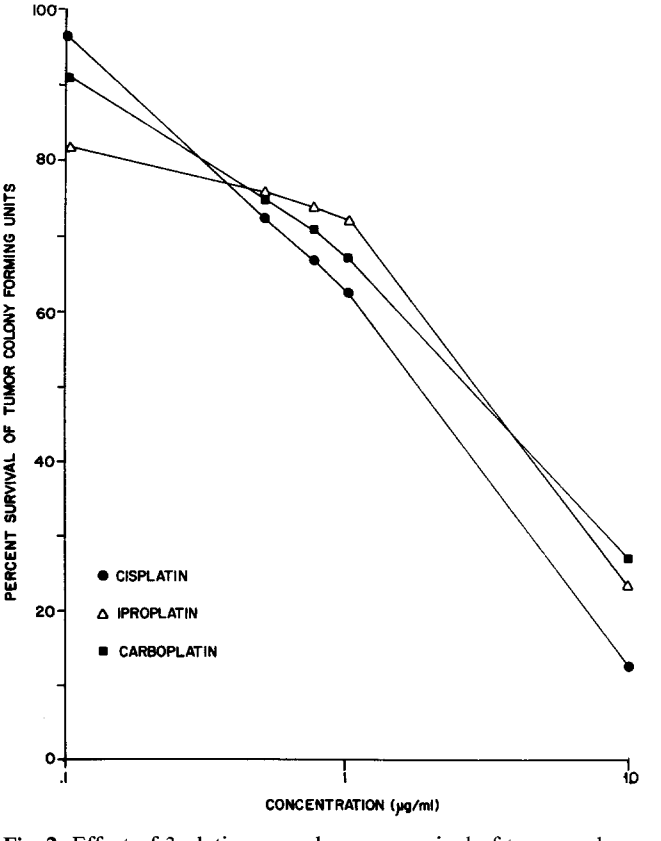

Fig. 2. Effect of 3 platinum analogs on survival of tumor colony-forming units (TCFUs) of UACC-66 ovarian cancer cell line following a 1-h exposure at 37° C. Experiments are duplicated. Each *point* represents the mean percentage survival of TCFUs

Table 1. Fraction of UACC-66 TCFUs[a] inhibited by heat, drugs: cisplatin, carboplatin, and iproplatin, and heat-drug combinations

Hyperthermia		Chemotherapy drug dose (μg/ml)			Fraction of UACC-66 TCFUs inhibited
T[b] (°C)	Heat dose (°C)	Cisplatin	Carboplatin	Iproplatin	
38.5	1.5				0.062
40	3				0.232
41.5	4.5				0.539
43	6				0.692
37	0	0.1			0.036
37	0	0.5			0.276
37	0	0.75			0.332
37	0	1			0.375
37	0	10			0.874
40	3	0.5			0.680
41.5	4.5	0.75			0.874
43	6	1			0.955
37	0		0.5		0.250
37	0		0.75		0.290
37	0		1		0.328
37	0		10		0.727
40	3		0.5		0.536
41.5	4.5		0.75		0.770
43	6		1		0.934
37	0			0.1	0.179
37	0			0.5	0.240
37	0			0.75	0.260
37	0			1	0.278
37	0			10	0.765
40	3			0.5	0.502
41.5	4.5			0.75	0.743
43	6			1	0.947

[a] Tumor colony-forming units
[b] Temperature

Table 2. Temperature and platinum analogue doses associated with inhibition of 0.5, 0.7, and 0.9 of UACC-66 TCFUs[a]

Fraction of UACC-66 TCFUs	Heat T[b] (°C)	Cisplatin (μg/ml)	Carboplatin (μg/ml)	Iproplatin (μg/ml)	Heat + cisplatin (6:1)[c]		Heat + carboplatin (6:1)[c]		Heat + iproplatin (6:1)[c]	
					T (°C)	μg/ml	T (°C)	μg/ml	T (°C)	μg/ml
0.50	41.6	1.596	2.564	2.611	39.4	0.403	40	0.495	40.1	0.521
0.70	43.5	3.492	8.475	10.594	40.1	0.521	40.8	0.629	40.9	0.642
0.90		12.151	56.949	98.669	41.7	0.786	42.5	0.920	42.4	0.896

[a] Tumor colony-forming units
[b] Temperature
[c] Heat dose (T - 37) at °C: combining drug dose in μg/ml

Results

To utilize the median-effect principal in the evaluation of cytotoxic additivity between hyperthermia and each of the three platinum analogues, it was first necessary to construct dose-survival curves for hyperthermia and each agent alone against the ovarian cancer cell line UACC-66. These dose-survival curves for hyperthermia and the three platinum analogues are shown in Figs. 1 and 2, respectively. Based on the curves in Fig. 2, the ID_{50} concentrations for cisplatin, carboplatin, and iproplatin were calculated to be 1.59, 2.56, and 2.61 μg/ml (1-h exposure), respectively. The temperature associated with 50% inhibition of tumor colony-forming units (TCFUs) from the UACC-66 cell line was 41.6° C.

It should be noted that there is a difference between temperature and heat doses. If a temperature of 37° C is equated to a starting heat dose of 0° C, then the temperatures of 38.5°, 40°, 41.5°, and 43° C correspond to the heat doses of 1.5°, 3°, 4.5°, and 6° C, respectively. When these heat doses (° C) were divided by relative combining drug doses (μg/ml), a constant combination ratio of 6:1 was determined. Table 1 shows the fraction of UACC-66 TCFUs inhibited by heat alone, each platinum analogue, and various heat-drug combinations. Based on these data, it was possible to compute the heat, drug, and heat-drug combination (using the 6:1 ratio) doses associated with inhibition of 50%, 70%, and 90% of the ovarian TCFUs (Table 2). At temperatures above 37° C, 50%–70% inhibition of UACC-66 TCFUs could be achieved with platinum anal-

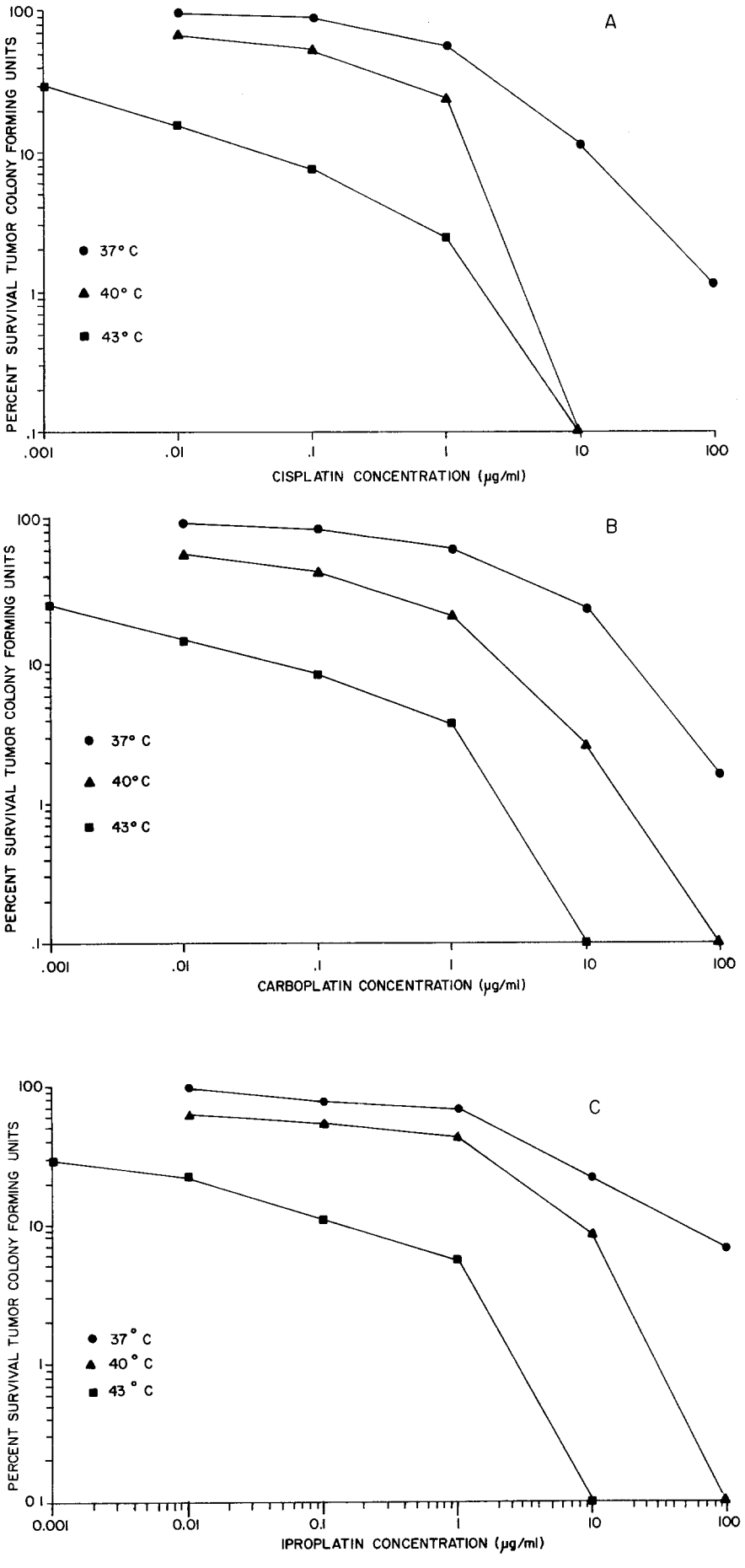

Fig. 3A–C. Thermal enhancement of UACC-66 tumor colony-forming unit inhibition by a 1-h treatment with each of three different platinum analogues (**A** cisplatin, **B** carboplatin, **C** iproplatin). The final concentrations tested ranged from 0.01 to 100 μg/ml for 37° C and 40° C, and 0.001 to 10 μg/ml for 43° C. Five-log dose-response curves were performed in duplicate for each temperature level

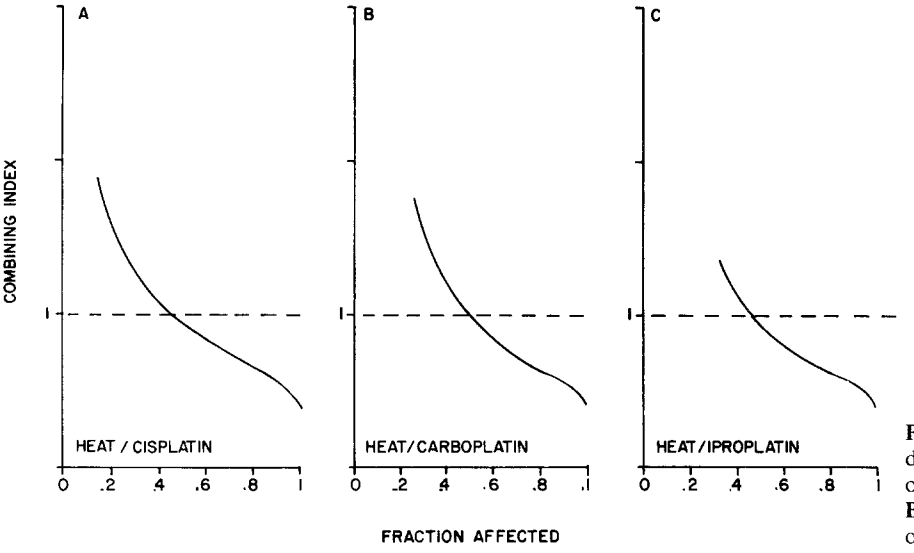

Fig. 4 A–C. Computer-generated median dose-effect plots of hyperthermia plus each of three platinum analogues (**A** cisplatin, **B** carboplatin, **C** iproplatin) against UACC-66 ovarian tumor colony-forming units

ogue concentrations of only 5%–25% of those required for the same cell kill at the control temperature of 37° C. It should also be noted that a temperature of only 40° C (1-h exposure) was sufficient to inhibit 50%–70% of TCFUs when combined wih each of the three drugs at concentrations that are achieved easily following standard clinical doses.

Figure 3 shows dose-survival curves for carboplatin and iproplatin combined with 1-h exposure to 37° C, 40° C, and 43° C against UACC-66 ovarian tumor clongenic cells. For each drug, when combined with heat, a 3° C incremental increase in temperature (i.e., from 37° C to 40° C, or from 40° C to 43° C) was associated with a tenfold decrease in ID_{50} drug concentration.

The results of the computer analysis of heat-drug combinations using the median-effect principle equations are shown in Fig. 4. With a heat increment-to-drug concentration ratio of 6:1, the shapes of the three curves reveal a synergistic interaction between heat and each of the three platinum analogues. In each case, the synergistic effect occurred when more than 40% of the tumor colony-forming units were inhibited.

Discussion

Patients with advanced ovarian cancer who have evidence of small-volume residual disease following second-look surgery still have a high likelihood of experiencing progressive disease and death even when further treatment is given subsequently. Recently, two independent studies of cisplatin administered by the i.p. route in such patient populations have resulted in a 33% pathologically proven complete response rate documented at third-look surgery [9, 19]. Because most patients receive between 400 and 600 mg/m^2 cisplatin as part of their initial chemotherapy, subsequent doses of cisplatin must be moderated to avoid severe neuropathy or nephropathy. Since response to treatment is closely associated with intensity of dose administered [16], the necessity to reduce cisplatin dose for retreatment severely limits the agent's efficacy.

Carboplatin and iproplatin have proven clinically useful when administered i.v. to patients with recurrent ovarian cancer [5, 13, 21, 24]. Although the doses of these agents are limited by myelosuppression, but not by neurotoxicity or nephrotoxicity, either may prove especially useful in the patient who has received relatively high doses of cisplatin and whose tumor may continue to be sensitive to platinum analogues.

We and other investigators have shown that cisplatin, carboplatin, and iproplatin have similar activities against human ovarian cancers in vitro [2, 3]. These three agents were virtually identical with respect to the sensitivity rate (i.e., percentage of tumors associated with an achievable ID_{50} concentration) of 45 ovarian cancers obtained at the time of initial surgery [2]. The slopes of the dose-survival curves for each of these three agents against our ovarian cancer cell line, UACC-66, demonstrate comparable activity for the three compounds; however, according to ID_{50} values (Table 2), cisplatin was slightly more potent than iproplatin, which was slightly more potent than carboplatin. Of course, the antitumor activities of these three drugs are not completely interchangeable; however, carboplatin, and to some degree iproplatin, appear to be relatively cross-resistant with cisplatin.

Cisplatin might appear to be an ideal drug to combine with hyperthermia, because incremental increases in the degree of hyperthermia are associated with incremental increases in the cytotoxicity of this agent [15]. Thus, even at 40° C, there is evidence of potentiation of cisplatin antitumor activity. Unfortunately, cisplatin's nephrotoxicity and neurotoxicity are also potentiated incrementally with increasing degrees of hyperthermia. The conclusions of one clinical trial were that systemic hyperthermia could not be combined safely with i.v.-administered cisplatin because of the high risk of life-threatening nephropathy [14]. The relative lack of both neurotoxicity and nephrotoxicity of carboplatin and iproplatin make them safer candidates for combination with hyperthermia. Our data concerning the synergistic effects of both of these agents with hyperthermia at all temperatures above 37° C provide a rationale for the design of clinical trials to test these hyperthermia-drug combinations in patients with ovarian cancer. Of course, severe myelosuppression resulting from the interaction of hyperthermia and these two platinum analogues could limit their clinical utility.

The mechanism of interaction between hyperthermia and the three platinum analogues has not been evaluated in

this study. There are published data showing that hyperthermia enhances the tumor cell uptake of cisplatin in a mouse tumor model [1]; however, it is likely that hyperthermia may directly or indirectly increase DNA intrastrand crosslinking by the platinum analogues.

There is increasing interest in the use of both carboplatin and iproplatin by the i.p. route. Recent reports suggest that these agents can be given in doses similar to those administered by the i.v. route and with only mild to moderate myelosuppressive effects [12, 17, 22] (J. H. Dorashow 1987, personal communication). Now that it is possible to heat the i.p. space using modern hyperthermia techniques, protocols designed to combine regional hyperthermia with i.p. drug administration have become feasible. Because of their relative safety and similar efficacies, both of the new platinum analogues appear promising for combination with heat in the setting of regional therapy for ovarian cancer.

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