Cancer Chemother Pharmacol (1996) 37: 574–580

ORIGINAL ARTICLE

G.P. Raaphorst · S. Doja · L. Davis · D. Stewart
C.E. Ng

A comparison of hyperthermia cisplatin sensitization in human ovarian carcinoma and glioma cell lines sensitive and resistant to cisplatin treatment

Received: 10 March 1995/Accepted: 21 July 1995

Abstract Two pairs of human tumor cell lines (glioma and ovarian carcinoma (OvCa)) each having a parental cell line and cisplatin-resistant variant, were evaluated for (a) cisplatin response, (b) hyperthermia response, and (c) combined hyperthermia and cisplatin response. The two resistant lines had comparable resistant responses while for the parental lines, the OvCa was more sensitive than the glioma to cisplatin doses up to 14 μg/ml. For the hyperthermia response, the OvCa parental line was more resistant than the variant line at low-temperature hyperthermia (41° C or 42°C) but became more sensitive at high temperature (45°C). For the glioma, the parental line was more sensitive to hyperthermia at all temperatures tested. Hyperthermia caused sensitization to cisplatin in all cell lines but was generally greater in the glioma cell lines. In the OvCa system, hyperthermia had a slightly greater sensitizing effect on the resistant cell lines, while in the glioma the opposite was true. The degree of sensitization increased with hyperthermia temperature. In summary, the results showed that there is no cross-resistance for hyperthermia and cisplatin, that the degree of thermal sensitization is not reduced in cisplatin-resistant cell lines, and that cisplatin thermal sensitization is cell-line and temperature dependent. Thus, hyperthermia can effectively improve tumor cell response to cisplatin and may be useful in overcoming resistance to cisplatin.

Key words Cisplatin · Hyperthermia · Thermal sensitization

Introduction

Hyperthermia has been investigated as an adjunct treatment for both radiotherapy and chemotherapy. Hyperthermia can cause an increased response (chemosensitization) to a wide range of chemotherapeutic agents (reviewed in reference 8). Several studies have shown that hyperthermia can cause cisplatin chemosensitization, and both synergistic and additive effects have been observed [4, 8, 9, 11, 17, 28]. It has been found that the effects of combined treatments are sequence dependent. Simultaneous treatment gives the greatest effect but drug treatment after hyperthermia is also effective [2, 5, 23, 26].

Cisplatin plays an important role in chemotherapy, but development of tumor drug resistance is a common cause of cisplatin treatment failure. Potential mechanisms of cellular cisplatin resistance include decreased drug uptake, enhanced removal of DNA adducts, increased levels of GSH, increased metallothioneins, and increased repair of DNA damage [3, 6, 13, 21, 22, 27].

Several studies suggest that adjuvant hyperthermia may be useful in overcoming resistance to cisplatin [7, 8, 12, 15, 20], although the degree of thermochemosensitization remains unclear. In some studies, the thermal enhancement ratios (TERs) were greater in cisplatin-resistant cells than in sensitive cells, while in other studies the opposite was true. In addition, cross-resistance between cisplatin and heat has been reported [7, 12, 15], but results of studies comparing heat and cisplatin resistance are scarce. Further studies have also shown that the cellular response to cisplatin is cell-cycle dependent, being greater in the G_1 than S phase [16], while other studies have shown different responses to cisplatin and/or hyperthermia in exponentially growing cells compared to confluent and spheroidal cultures [1, 17]. In a recent study on the effects of hyperthermia at 42°C on cisplatin sensitization, we have shown that both cisplatin-sensitive and resistant cells are more sensitive to cisplatin treatment during the plateau phase than exponentially growing cells [24].

In this study, we set out to evaluate the effect of a wide range of hyperthermia temperatures on cisplatin sensitization in human ovarian carcinoma cells and

G.P. Raaphorst (✉) · S. Doja · L. Davis · D. Stewart · C.E. Ng
Medical Physics Department, Ottawa Regional Cancer Centre, 190 Melrose Avenue, Ottawa, Ontario, K1Y 4K7, Canada

glioma cells in both normally responding and in cisplatin-resistant variants. The aims of the study were: (1) to determine whether thermal cisplatin sensitization can be achieved at clinically relevant temperatures, (2) to determine whether thermosensitization is temperature dependent, (3) to determine whether thermosensitization can be achieved in cisplatin-resistant variants, and (4) to compare sensitization in the variants and their parental cell lines over a wide temperature range. For these experiments, cells in the plateau phase were used to avoid treatment-related cell-cycle redistribution and also because we had found cells in this phase to be more sensitive to cisplatin than those in the exponential growth phase.

Materials and methods

The cell line U373MG was originally established from a human malignant astrocytoma [29]. The cisplatin-resistant variant U373MG^{CP} was developed in our laboratory by chronic exposure of the parental U373MG to stepwise increasing concentrations of cisplatin, then selection of a surviving clone following a challenge dose of cisplatin.

The cell line A2780 is a human ovarian carcinoma cell line originally derived from an untreated patient [10]. The A2780^{CP} is a cisplatin-resistant variant obtained by chronic exposure of A2780 to stepwise increasing concentrations of cisplatin up to 12 μg/ml [18].

Cells were grown in a 1:1 mixture of DMEM and F-12 medium, supplemented with 7.5% fetal bovine serum, 7.5% newborn calf serum, 0.1 m*M* MEM non-essential amino acids, 10 m*M* sodium bicarbonate, and 20 m*M* HEPES, and incubated at 37°C in a humidified atmosphere of 2% CO_2 and 98% air. Experiments were done with cells grown into the plateau phase. For the plateau phase, cells were fed with fresh medium 2 days before the experiment and the plating efficiencies were in the ranges 30–35%, 50–55%, 18–25%, and 30–40% for the A2780, 2780^{CP}, U373MG and the U373MG^{CP} lines, respectively.

Cisplatin was obtained as Cisplatin Injection (David Bull Canada, Vaudreuil, PQ, Canada) consisting of 1 mg/ml (3.33 m*M*) cis-diaminedichloroplatinum(II) and 9 mg/ml NaCl, pH adjusted to 7.3. Cells were treated by adding a measured amount of this solution directly to the culture medium covering the cells. At the end of the exposure period, the medium containing cisplatin was aspirated, and cells were rinsed twice with isotonic citrate saline and new medium was added.

Hyperthermia treatments were performed by sealing T-25 flasks with parafilm and immersing them in a circulating water bath with temperature controlled to $\pm$ 0.05°C. Flasks were placed in a 37°C water bath for 2 min at the end of the hyperthermia treatment period.

Cell survival after treatment was determined by a colony-forming assay. Briefly, cells were rinsed with isotonic citrate saline, trypsinized (0.2% w/v trypsin in citrate saline for 5 min at 37°C), counted using an electronic cell counter, and plated into 60-mm dishes containing fresh medium. Colonies larger than 50 cells at days 10, 14, and 20 (A2780 and 2780^{CP}, U373MG, and U373MG^{CP} respectively) were scored as survivors. Plotted points represent the mean of three replicate experiments. Error bars are standard error of the mean.

Results

The cisplatin response of the two pairs of cisplatin-resitant and -sensitive cell lines is shown in Fig. 1. For both the ovarian carcinoma and the glioma cell lines, the resistant lines had a much higher survival after cisplatin exposure than the normally responding parental lines. The ovarian carcinoma parental cell line (A2780) was more sensitive than the glioma parental cell line (U373MG) and the glioma resistant variant (U373MG^{CP}) and ovarian carcinoma resistant variant (A2780^{CP}) were approximately equally responsive. The

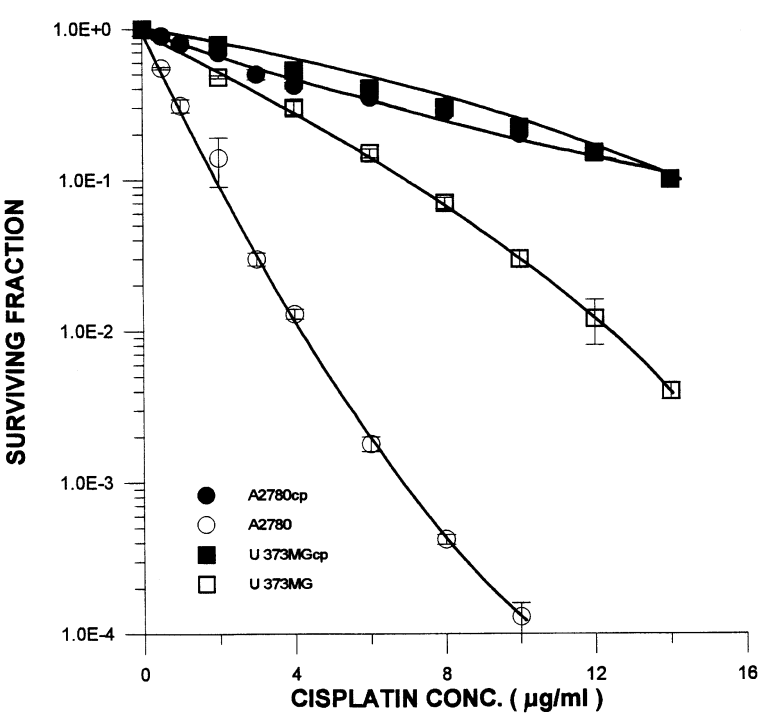

Fig. 1 The response of ovarian carcinoma and glioma cells to 1 h cisplatin treatment for cells sensitive and resistant to cisplatin

results imply that resistance may remove differences in response to cisplatin among cell lines.

The hyperthermia response of the four cell lines is shown in Figs. 2–4. For the ovarian carcinoma cell lines the parental cell line was more hyperthermia resistant than the variant for the lower temperature hyperthermia treatments. At the higher temperature (45°C) and for longer heating times (43°C, 44°C) the relative thermal sensitivity was reversed and the parental cell line became more resistant than the variant. For the glioma cell lines (Fig. 4) the parental cell line was more sensitive to the three temperatures tested than the cisplatin-resistant variant. This difference in thermal response increased with temperature and was in excess of

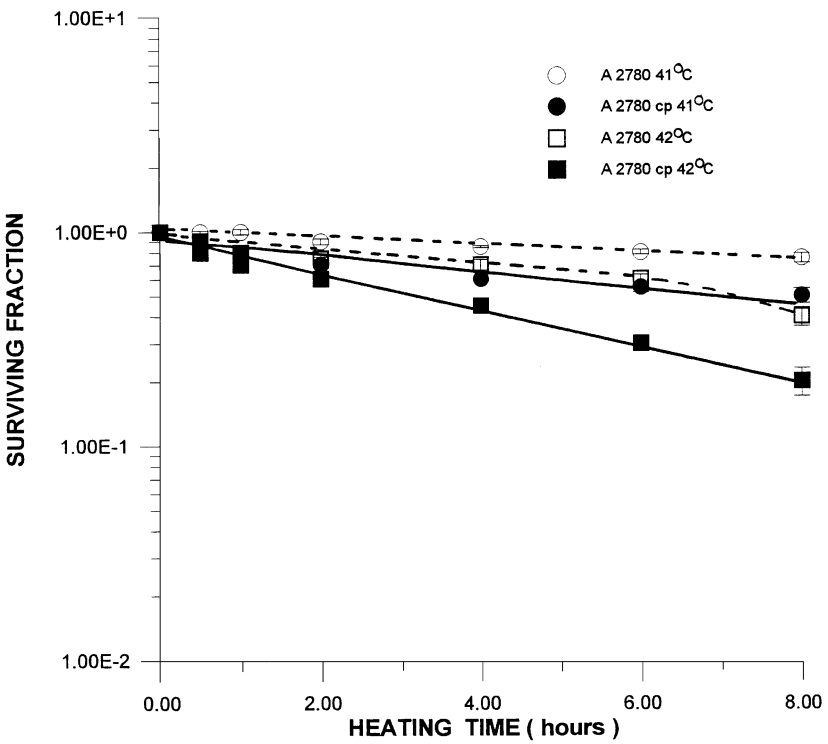

Fig. 2 The response of ovarian carcinoma cells to hyperthermia for cells sensitive and resistant to cisplatin

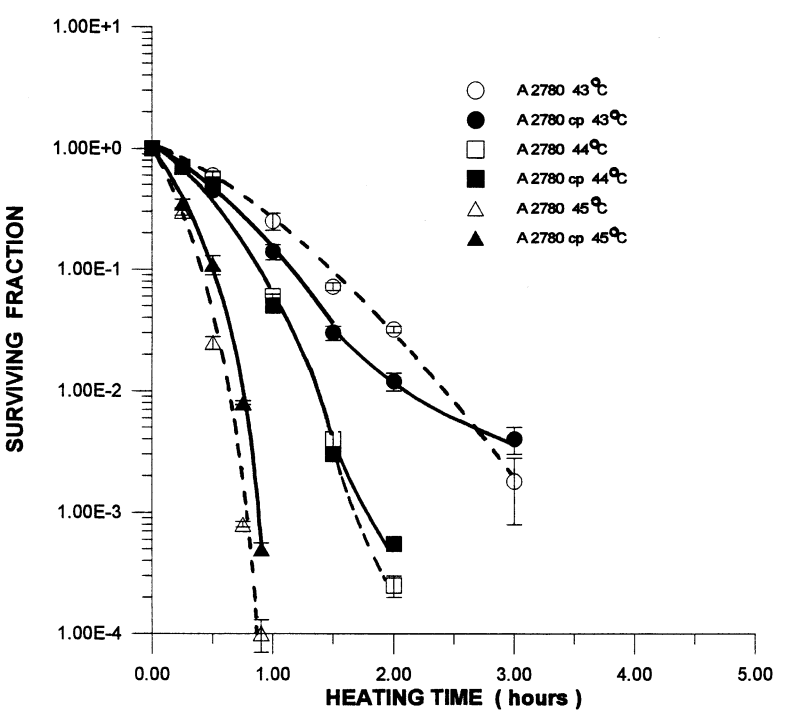

Fig. 3 The response of ovarian carcinoma cells to hyperthermia for cells sensitive and resistant to cisplatin

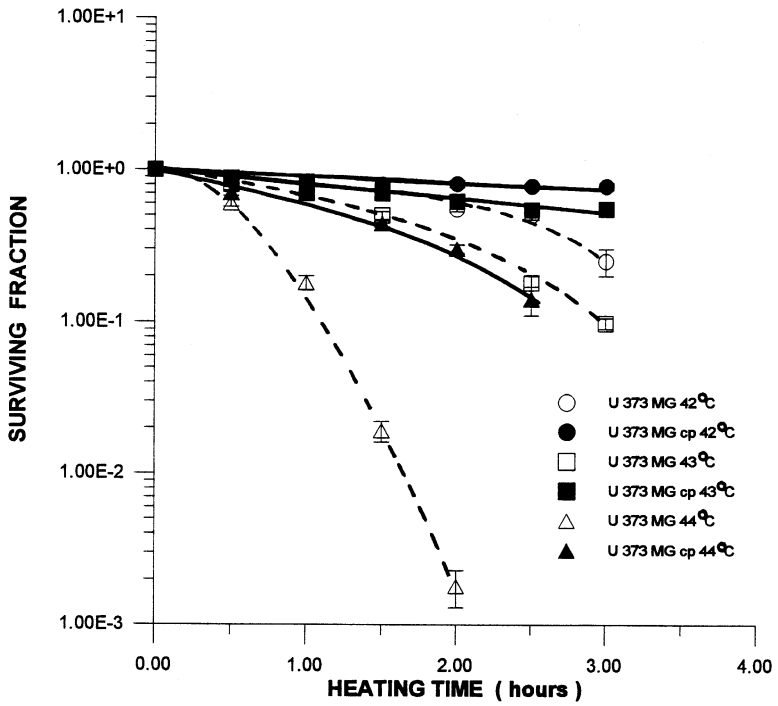

Fig. 4 The response of glioma cells to hyperthermia for cells sensitive and resistant to cisplatin

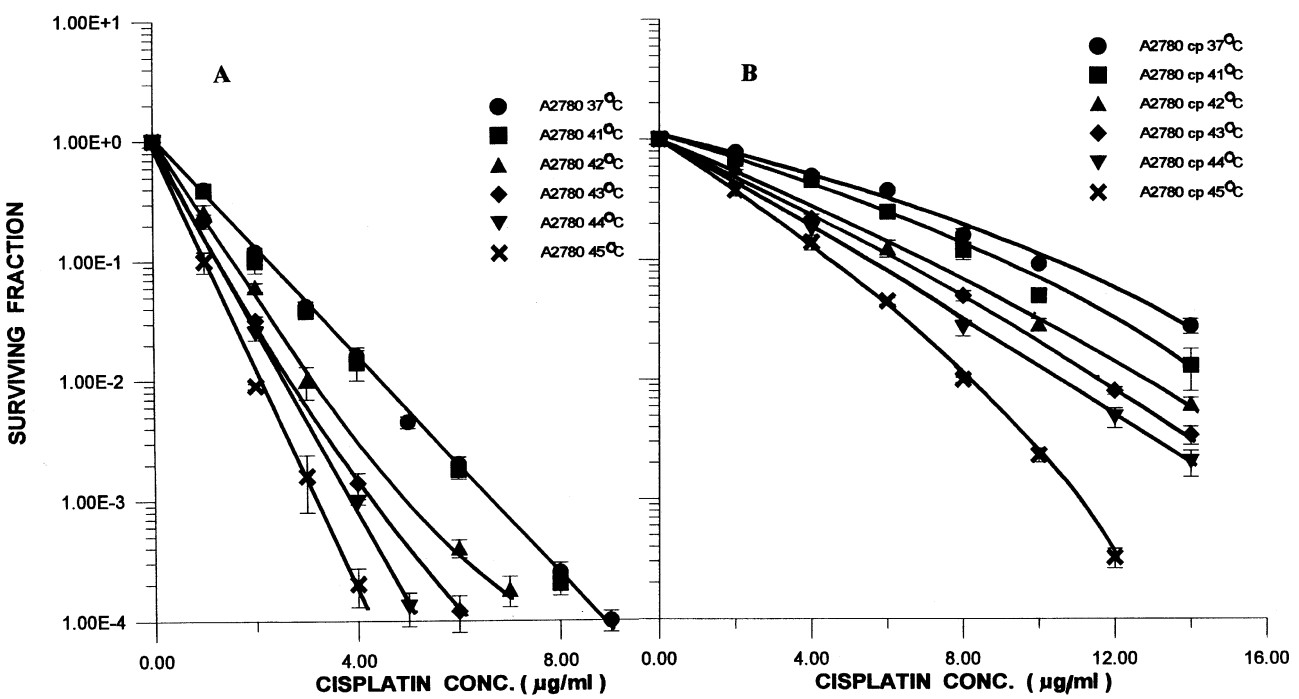

Fig. 5A, B The response of cisplatin-sensitive (**a**) and cisplatin-resistant (**b**) ovarian carcinoma cells to hyperthermia and cisplatin given concurrently for 1 h

a 100-fold difference in survival after heating for 2 h at 44°C.

The interaction of hyperthermia and cisplatin treatment was tested on all four cell lines. The cells were exposed to cisplatin and hyperthermia simultaneously for 1 h. For the ovarian carcinoma cell lines, hyperthermia resulted in sensitization to cisplatin treatment in both the parental and the resistant variant cell lines (Fig. 5). Table 1 shows the degree of sensitization at two survival levels. The TERs were calculated by dividing the dose of drug given at 37°C by the dose given at the hyperthermia temperature to achieve equal survival levels. Analysis was done at the 10% and 1% survival levels. For some of the values, the data had to be interpolated or extrapolated from the figures. The TER increased with hyperthermia temperature in both the sensitive and resistant cell lines (Table 1). The TERs did not depend on the survival level at which they were

Table 1 Thermal sensitization to cisplatin treatment. Values are thermal enhancement ratios calculated by dividing the dose of cisplatin at 37°C to obtain the 10 or 1% survival levels by the dose of cisplatin at hyperthermia temperatures to obtain the same survival levels

	A2780				U373MG			
	Sensitive		Resistant		Sensitive		Resistant	
Temperature (°C)	10%	1%	10%	1%	10%	1%	10%	1%
41	1.00	1.00	1.14	1.19	2.50	2.10		
42	1.29	1.34	1.44	1.39			1.39	1.37
43	1.57	1.53	1.55	1.51	3.50	3.39	2.18	2.28
44	1.57	1.65	1.85	1.71	4.38	3.94	2.48	2.41
45	2.10	2.05	2.13	2.15				

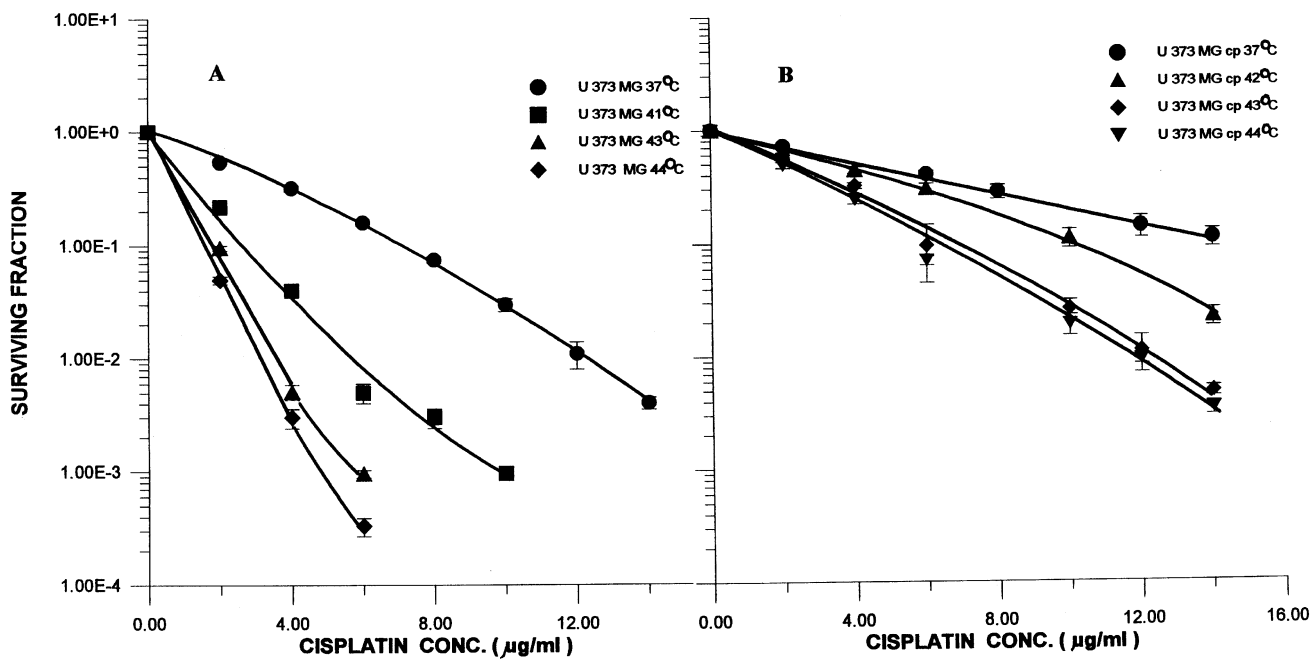

Fig. 6A, B The response of cisplatin-sensitive (**a**) and cisplatin-resistant (**b**) glioma cells to hyperthermia and cisplatin given concurrently for 1 h

calculated. The TERs were higher in the resistant ovarian carcinoma cell line than in the sensitive cell line, but the differences were not large and were only significant in a few cases.

For the glioma cell lines shown in Fig. 6, thermal sensitization was achieved in both the parental and the variant lines. The TERs (shown in Table 1) were comparable at the 10% and 1% survival levels. Increases in the TERs were observed as the temperature was increased. The TERs were higher in the sensitive cell line than in the resistant variant. In addition, the TERs were generally higher in the glioma cell lines than in the ovarian carcinoma cell lines.

Discussion

It has been reported previously that hyperthermia can cause sensitization to cisplatin in mammalian cells [4, 8, 9, 11, 14, 17, 28]. In some studies, the effects were synergistic, while in others, the results were additive and, indeed, the induction of heat shock proteins may even induce a slight level of antagonism [14]. However, different temperatures were used in the various studies and the temperature dependence remains unclear.

In this study, we evaluated the effects of hyperthermia treatment for 1 h over a temperature range of 41–45°C in four human tumor cell lines. For the ovarian carcinoma cell lines, we found that the thermal sensitization increased with temperature giving TERs ranging from 1.0 (no effect) at 41°C to 2.15 at 45°C (a large effect). It should be noted that at the clinically achievable temperatures of 42°C and 43°C, the TER was significant, ranging from 1.29 to 1.57 (Table 1). For the glioma cell lines, the TER also increased with temperature. In general, the thermal sensitizing effect was larger in the glioma cell lines than in the ovarian carcinoma lines. Even though hyperthermia sensitization has already been demonstrated in glioma and ovarian carcinoma cell lines in different studies, the results cannot be compared because different temperatures were used and the cell lines were from different

mammals (rat glioma and human ovarian carcinoma) [20, 25]. In our studies, using the same cell culture conditions and same temperatures, we were able to show that there can be significant differences in thermosensitization between cell lines such as glioma and ovarian carcinoma. Although cell cultures may not respond in the same way as tumors, these results indicate that the degree of thermal sensitization to cisplatin may not be the same for cells of different origins, and may have to be examined for each potential tumor type to be treated with this thermochemotherapy regimen.

It is well documented that mammalian cells can become resistant to cisplatin treatment. Investigations on overcoming this resistance have led to the testing of hyperthermia as a cisplatin sensitizer in cisplatin-resistant cells. Several studies have shown for one or two temperatures that hyperthermia can cause sensitization in resistant cells but the results are contradictory. Some studies have shown that the thermal sensitization is about the same in resistant and sensitive cell lines [12], and others have shown that it is less in resistant cell lines than in sensitive lines [20] or that it is greater in resistant lines than in sensitive lines [7, 15]. In these studies, temperatures of 42°C and 43°C were used. It has also been shown that cisplatin-resistant cells have higher [19], the same [12] or lower [15] heat sensitivity than sensitive cells. Thus, the results regarding cross-resistance for cisplatin and hyperthermia and thermal cisplatin sensitization in cisplatin-sensitive versus-resistant cells are ambiguous. We and others have shown that the degree of thermal sensitization is cell-cycle and cell growth-phase dependent [16, 24] and the variation amongst the various studies may in part be related to cell-cycle differences, as well as to the choice of different temperatures and tissue culture conditions. Thus, we studied all our cell lines during the same growth phase (plateau phase), using the same cell culture conditions and comparing the same hyperthermia temperatures.

Our results show that there is no single pattern for cross-resistance or sensitivity to hyperthermia and cisplatin. For example, in the glioma cells the parental cell line was more thermally sensitive than the cisplatin-resistant variant, while in the ovarian carcinoma cells the parental cell line was more thermally resistant than the cisplatin-resistant variant except at the highest temperature (45°C), where the opposite was true.

For thermal sensitization to cisplatin toxicity, there was also no consistent trend between the two pairs of tumor cell lines. For example, in the ovarian carcinoma cells, the thermal sensitization was greater in the cisplatin-resistant line than in the parental sensitive line. In the glioma system, the opposite was true. However, it should be noted that significant thermal sensitization was achieved in all cell lines at temperatures that are clinically achievable.

In summary, we have shown that there is no cross-resistance for hyperthermia and cisplatin, that the degree of cisplatin sensitization by hyperthermia is not reduced in cisplatin-resistant tumor cell lines and that thermal cisplatin sensitization is temperature and cell-line dependent. This cell line/type dependence may indicate that the efficacy of combined therapy may be tumor site dependent and further investigation is warranted.

Acknowledgement This work was supported by a grant from NCI Canada.

References

1. Boitrel MTD, Pelletier H, Genne P, Petit JM, Le Grimellec C, Canal P, Ardiet C, Bastian G, Chauffert B (1992) Confluence-dependent resistance in human colon cancer cells: role of reduced drug accumulation and low intrinsic chemosensitivity of resting cells. Int J Cancer 50:677
2. Bowden GT, Kasunic M, Dim D (1983) Sequence dependence for the hyperthermic potentiation of cis-diaminedichloroplatinum (II) induced cyctotoxicity and DNA damage (abstract). Proceedings Third North American Hyperthermia Group, p 161
3. Canon JL, Humblet Y, Symann M (1990) Resistance to cisplatin: How to deal with the problem? Eur J Cancer 26:1
4. DaSilva VF, Raaphorst GP, Goyal R, Feeley MM (1987) Drug cytotoxicity at elevated temperature. J Neurosurg 67:83
5. Douple EB, Strohbehn JW, deSieyes DC, Alborough DP, Trembly BS (1982) Therapeutic potentiation of *cis*-diamminedichloroplatinum (II) and radiation by interstitial microwave hyperthermia in a mouse tumour. Natl Inst Drug Abuse Res Monogr Ser 61:259
6. Eastman A, Schulte N (1988) Enhanced DNA repair as a mechanism of resistance to cis-diaminedichloroplatinum (II). Biochemistry 27:4730
7. Eichholtz-Wirth H, Hietel B (1990) Heat sensitization to cisplatin in two cell lines with different drug sensitivities. Int J Hyperthermia 6:47
8. Engelhardt R (1987) Hyperthermia and drugs. Recent Results Cancer Res 104:136
9. Hahn GM (1979) Potentiation of therapy drugs and hyperthermia. Cancer Res 39:2264
10. Hamilton TC, Lai GM, Rothenberg LM, Fojo AT, Young RC, Ozols RF (1989) Mechanisms of resistance to cisplatin and alkylating agents. In: Ozols RF (ed) Drug resistance in cancer therapy. Kluwer Academic Publishers, Boston page 151–169
11. Herman TS, Sweets CC, White DM, Gerner EW (1982) Effect of rate of heating on lethality due to hyperthermia and selected chemotherapy drugs. J Natl Cancer Inst 68:487
12. Herman TS, Teicher BA, Cathcart FNS, Kaufman ME, Lee JB, Lee M (1988) Effect of hyperthermia on cisdiaminedichloroplatinum (II) and pt(Rh-123) in a human squamous carcinoma cell line and a cisplatin resistant subline. Cancer Res 48:5101
13. Jekunen AP, Hom DK, Alcaraz JE, Eastman A, Howell SB (1994) Cellular pharmacology of dichloro(ethylenediamine) platin II in cisplatin-sensitive and resistant human ovarian carcinoma cells. Cancer Res 54:2680
14. Kimura E, Howell SB (1993) Analysis of the cytotoxic interaction between cisplatin and hyperthermia in human ovarian carcioma cell line. Cancer Chemother Pharmacol 32:419
15. Konings AWT, Hettinga JVE, Lemstra W, Humphrey GB, Kapinga HH (1993) Sensitizing for cisplatin action by hyperthermia in resistant cells. Int J Hyperthermia 9:553
16. Krishnaswamy G, Dewey WC (1993) Cell killing and chromosomal aberrations induced in Chinese hamster ovary cells by

treating with cisplatin at 41.5°C during G_1 or late S phase. Cancer Res 53:1239
17. Kubota N, Kakehi M, Inada T (1993) Hyperthermic enhancement of cell killing by five platinum complexes in human malignant melanoma cells grown as monolayer cultures and multicellular spheroids. Int J Radiat Oncol 25:491
18. Lai GM, Ozols RF, Smyth JF, Young RC, Hamilton TC (1988) Enhanced DNA repair and resistance to cisplatin in human ovarian cancer. Biochem Pharmacol 37:4597
19. Mansouri A, Henle KJ, Benson AM, Moss AJ, Nagle WA (1989) Characterization of a cisplatin-resistant subline of murine RIF-1 cells and reversal of drug resistance by hyperthermia. Cancer Res 49:2674
20. Masuda H, Tanaka T, Kido A, Kitamura K, Kusaba I (1991) Hyperthermic enhancement of cytotoxicity and DNA-binding of cisplatin in sensitive and resistant human ovarian cancer cell lines. Cancer J 4:283
21. Meijer C, Mulder NH, Hospers GAP, Uges DRA, de Vries EGE (1990) The role of glutathione in resistance to cisplatin in a human small cell lung cancer cell line. Br J Cancer 62:72
22. Micetich K, Zwelling L, Kohn KW (1983) Quenching of DNA:platinum (II) monoadducts as a possible mechanism of resistance to cis-diaminedichloroplatinum (II) in L1210 cells. Cancer Res 43:3609
23. Miyahara T, Ueda K, Akaboshi M, Shimada Y, Imamura M, Utsumi H (1993) Hyperthermic enhancement of cytotoxicity and increased uptake of cis-diaminedichloroplatinum (II) in cultured human esophageal cancer cells. Jpn J Cancer Res 84:336
24. Raaphorst GP, Chabot P, Doja S, Wilkins D, Stewart D, Ng CE (1995) Effect of hyperthermia on cisplatin sensitivity in human glioma and ovarian carcinoma cell lines resistant and sensitive to cisplatin treatment. Int J Hyperthermia (in press)
25. Schen BC, Mella O, Dohl O (1992) Thermochemotherapy with cisplatin or carboplatin in the BT_4 rat glioma in vitro and in vivo. Int J Radiat Oncol 23:109
26. Supi G, Badaracco G, Cavatiere R, Natali FG, Gerco C (1994) Influence of sequence on hyperthermia and drug combination. Hyperthermic Oncol I:429
27. Tashiro T, Sato Y (1992) Characterization of acquired resistance to cis-diaminedichloroplatinum (I) in mouse leukemia cell lines. Jpn J Cancer Res 83:219
28. Urano M, Kahn J, Kenton LA (1990) The effect of cisplatin I treatment at elevated temperatures on murine fibrosarcoma. F.Sa-II. Int J Hyperthermia 6:563
29. Westermark B, Ponten J, Hugossen R (1973) Determinants for the establishment of permanent tissue culture lines from human gliomas. Acta Pathol Microbiol Scand [A] 81:791