Effect of 41˚C and 43˚C on cisplatin radiosensitization in two human carcinoma cell lines with different sensitivities for cisplatin

JUDITH W.J. BERGS, JAAP HAVEMAN, ROSEMARIE TEN CATE, JAN PAUL MEDEMA, NICOLAAS A.P. FRANKEN and CHRIS VAN BREE

Academic Medical Center, Laboratory for Experimental Oncology and Radiobiology (LEXOR), Department of Radiotherapy, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands

Received January 26, 2007; Accepted March 2, 2007

Abstract. The effect of trimodality treatment consisting of hyperthermia, cisplatin and radiation was investigated in two cell lines with different sensitivities to cisplatin. Hyperthermia treatment was performed for 1 h at 41˚C and 43˚C in order to compare the effects of the two temperatures. Clonogenic assays were performed with cisplatin-sensitive SiHa human cervical carcinoma and cisplatin-resistant SW-1573 human lung carcinoma cell lines. Cells were treated with various combinations of hyperthermia, cisplatin and radiation. Radiation was performed after 1 h of simultaneous hyperthermia and cisplatin treatment. Cisplatin exposure was for 1 h or continuous without refreshment of the cisplatin-containing medium. SiHa cells were more sensitive to cisplatin than SW-1573 cells. Hyperthermia at 41˚C decreased survival in SW-1573 cells but was not cytotoxic in SiHa cells. Hyperthermia at 43˚C decreased survival dramatically in both cell lines with SiHa being the most sensitive. The addition of hyperthermia at 41˚C and 43˚C to cisplatin treatment led to enhanced cell kill in both cell lines compared with cisplatin alone. Radiosensitization was observed after continuous but not after 1 h of cisplatin treatment. Hyperthermia at 43˚C increased radiosensitivity whereas hyperthermia at 41˚C did not. A combination of 41˚C hyperthermia with continuous cisplatin treatment had an additive effect on SW-1573 cells but enhanced cisplatin radiosensitivity of SiHa cells. In SW-1573 cells trimodality treatment using 43˚C hyperthermia enhances cisplatin radiosensitivity. We conclude that hyperthermia at 43˚C enhances cisplatin-induced radiosensitization in both cisplatin-sensitive and -resistant cell lines. Hyperthermia at 41˚C was also able to increase cisplatin-induced radiosensitivity but only in the cisplatin-sensitive SiHa cell line.

Correspondence to: Dr Judith W.J. Bergs, Academic Medical Center, LEXOR/Department of Radiotherapy, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands
E-mail: j.w.bergs@amc.uva.nl

Key words: cisplatin, hyperthermia, radiosensitization

Introduction

Hyperthermia refers to heat treatment of malignancies in which tumour temperature is elevated to the range of 39-45˚C. It is used in combination with chemotherapy and/or radiotherapy since it is known to enhance the anti-cancer effects of both therapies (1-3). Many *in vitro* studies on the combination of hyperthermia and radiation have shown a synergistic interaction between the two modalities, especially at higher temperatures (>42˚C) (4-13). This interaction is believed to result from inhibition of radiation-induced DNA damage repair by hyperthermia (14).

Cisplatin is a widely used chemotherapeutic agent which is often used in combination with hyperthermia or radiation (15-22). Its cytotoxicity has been shown to be enhanced by increasing temperatures. This effect might well be explained by the intracellular accumulation of cisplatin relative to the extracellular medium concentration as a result of the hyperthermia treatment (15). Cellular cisplatin uptake and the resulting cytotoxicity has been shown to increase when the incubation time is prolonged (15). Cisplatin has also been shown to cause radiosensitization (23,24). Therefore, intracellular cisplatin accumulation by hyperthermia may lead to increased radiosensitization when hyperthermia is added to cisplatin and radiation treatment. Increased cytotoxicity either by an additive effect or by increased radiosensitization has been observed in cells using trimodality treatment *in vitro* (25-27). In the clinic, trimodality treatment is currently tested in phase III trials in patients with advanced stage cervical carcinomas (28). In this trial, the trimodality treatment was compared with the conventional cisplatin and radiation treatment.

Despite the clinical goal to reach (cytotoxic) temperatures as high as 43˚C, tumour temperature distribution is heterogeneous. In large areas of the tumour, temperatures are often <43˚C (29). Nonetheless, good results have been obtained in locally advanced cervical cancers with tumour temperatures <43˚C (30). Mild temperatures have more subtle effects than high temperatures, such as tumour reoxygenation (29).

Many studies exist on the interaction of hyperthermia with cisplatin or radiation and of cisplatin with radiation. Most of these reports focus mainly on hyperthermia at temperatures >42˚C. However, data on trimodality treatment using clinically more relevant hyperthermia treatment at 41˚C are limited. Therefore, we compared the effect of 41˚C combined with cisplatin and radiation with that of 43˚C combined with cisplatin and radiation in this study. The extent of radiosensitization after hyperthermia and/or cisplatin was compared in two human cancer cell lines with different sensitivities for cisplatin. The SiHa cervical carcinoma cell line is sensitive to cisplatin and the SW-1573 lung carcinoma cell line is less sensitive. Cisplatin incubation was performed during the 1-h hyperthermia treatment with or without prolongation of the incubation period after the hyperthermia treatment.

Materials and methods

Cell culture. The human squamous cell lung carcinoma cell line SW-1573 was grown in Leibowitz-15 medium (Gibco-BRL Life Technologies, Breda, The Netherlands) supplemented with 10% heat-inactivated foetal bovine serum (FBS) and 2 mM glutamine and kept at 37˚C in a humidified air-filled incubator without additional CO_2. The SW-1573 cultures have a doubling time of ~24 h. The human cervical squamous cell carcinoma cell line SiHa was grown in Dulbecco's modified Eagle's medium with 4500 mg/l glucose (Gibco-BRL Life Technologies) supplemented with 10% heat-inactivated FBS and 2 mM glutamine and kept at 37˚C in a humidified incubator with a 10% CO_2/90% air atmosphere. The SiHa cultures have a doubling time of ~60 h. Both cell lines were maintained as monolayers in tissue culture flasks (Costar Europe Ltd., Badhoevedorp, The Netherlands). The cultures were passaged once or twice a week to ensure exponential growth.

Clonogenic assay. Clonogenic assays were conducted as described by Franken *et al* (31). For experiments, cells were trypsinized and seeded in appropriate dilutions for each treatment in 6-well plates (Costar Europe Ltd.). The cells were incubated at 37˚C for at least 3 h to accomplish attachment of the cells to the bottoms of the wells. Trypsinization prior to irradiation, hyperthermia and/or cisplatin incubation had no effect on the cellular sensitivity to these treatments because plating efficiencies and surviving fractions were not different from those of cells plated after treatment.

Irradiation treatments were performed with single doses of 2, 4, 6 and 8 Gy γ-rays from a ^{137}Cs source at a dose rate of about 0.7 Gy/min. Irradiation treatments were combined with cisplatin or hyperthermia or with both cisplatin and hyperthermia. Cisplatin (platosin®, Pharmachemie, Haarlem, The Netherlands) was diluted just before use in phosphate-buffered saline (PBS) from a freshly prepared 3.3-mM stock. In control cells only PBS was added.

To determine the sensitivity of both cell lines for cisplatin, cells were treated with doses of cisplatin from 1-100 μM and incubated for 1 h or continuously. A concentration of 5 μM has been shown to be the optimal steady-state plasma level in cancer patients (32). For the 1-h cisplatin incubation, the cisplatin-containing medium was removed, wells were washed with PBS and fresh medium was added to the cells. For the continuous cisplatin treatments, the cisplatin-containing medium was left on the cells until fixation without refreshment of cisplatin. Cisplatin sensitivity was indicated by the inhibiting concentration (IC10) which is the concentration needed to decrease survival to the 10% level and is shown in Table I.

The sensitivity of the cells for hyperthermia was determined by incubating the cells at 41 and 43˚C using a waterbath for 1-4 h. The atmosphere of the waterbath was adjustable by a connection with air and CO_2 supplies. SiHa cells were heated in a 10% CO_2/90% air atmosphere with an air inflow of 2 l/min. SW-1573 cells did not receive additional CO_2. After hyperthermia treatment, the cells were returned to a 37˚C incubator. Hyperthermia sensitivity was indicated by the inhibiting exposure time (IET50) which is the exposure time needed to decrease survival to the 50% level and is shown in Table II.

For combined hyperthermia and cisplatin treatments, cisplatin incubation and hyperthermia treatment were performed simultaneously for 1 h. For continuous cisplatin treatment the incubation was prolonged after hyperthermia treatment. The sensitizing effect of hyperthermia on cisplatin cytotoxicity was described by the thermal enhancement ratio (TER) at 10% survival. The TER was calculated by dividing the cisplatin dose necessary to decrease survival to 10% by the cisplatin dose necessary to decrease survival to 10% when combined with hyperthermia. TERs are shown in Table III.

The effect of cisplatin on radiosensitivity was studied by incubation with cisplatin for 1 h with concentrations of 1, 5 and 10 μM for both SiHa and SW-1573 cells and continuously with 1 and 5 μM for the SW-1573 cells and with 1 μM for SiHa cells. Higher concentrations were too toxic to obtain colonies (consisting of 50 cells or more). In addition, IC50 equitoxic cisplatin doses of 5.0 and 16.5 μM for 1 h and 0.4 and 3.6 μM continuously were applied for SiHa and SW-1573 cells, respectively.

To study the effect of hyperthermia treatment alone and in combination with cisplatin on the radiosensitivity of the cells, hyperthermia at 41˚C and 43˚C was performed in a waterbath for 1 h with or without simultaneous cisplatin treatment for 1 h or continuously. Cells treated for 1 h with combined hyperthermia and cisplatin were irradiated after the combination treatment after replacing the cisplatin-containing medium with fresh medium. For the SiHa cells, a mixture of 10% CO_2 in 2 l/min air was applied during the hyperthermia treatment. The radiation enhancement ratio (RER) was calculated by dividing the radiation dose necessary to decrease survival to 10% after radiation alone by the radiation dose necessary to decrease survival to 10% after combined treatment. These ratios are shown in Table IV.

γ-irradiation was performed after hyperthermia and after 1 h of cisplatin incubation because this method has been shown to obtain the largest effect on cytotoxicity (15). The SW-1573 cells were fixated 7-8 days after treatment in 6% glutaraldehyde and stained with 0.05% crystal violet. The SiHa cells were fixated 14 days after treatment because the doubling time (60 h) is higher than for the SW-1573 (24 h)

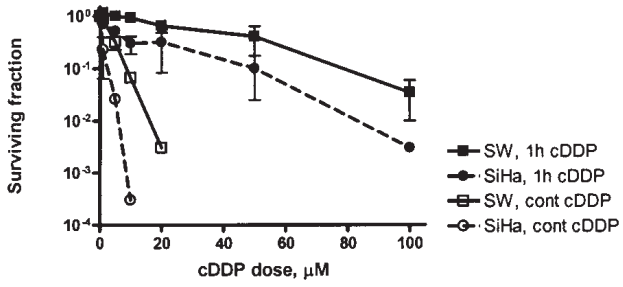

Figure 1. Survival of SW-1573 and SiHa cells after different concentrations of cisplatin exposure for 1 h and after continuous exposure. Means ± SEM are shown for at least three separate experiments.

Table I. Inhibiting concentration (IC10) after cisplatin treatment.

	IC10 values (μM)	
	One-hour treatment (SEM)	Continuous incubation (SEM)
SW-1573	74.5 (20.5)	8.7 (0.6)
SiHa	49.3 (20.8)	2.1 (0.9)

cells. Colonies of 50 cells or more were scored as originating from a single clonogenic cell.

Statistics. Data on cell survival after cisplatin, hyperthermia or combined cisplatin and hyperthermia treatment were analyzed using SPSS (Chicago, IL, USA) statistical software using the non-parametric Mann-Whitney test.

For radiation survival curves, the surviving fractions [S(D)/S(0)] after radiation dose (D) were corrected for the toxicity of cisplatin and hyperthermia alone or for combined cisplatin and hyperthermia toxicity. Statistical significance of differences between the survival curves was calculated using SPSS by weighted linear regression according to the linear-quadratic formula: $S(D)/S(0)=\exp-(\alpha D+\beta D^2)$ (http://www.amc.nl/index.cfm?sid=817).

Results

Cisplatin sensitivity. To determine the sensitivity of SW-1573 and SiHa cells for cisplatin, clonogenic survival was determined after a 1-h incubation and continuous incubation (Fig. 1). The results show that SiHa cells are more sensitive for cisplatin than SW-1573 cells. This difference was statistically significant for continuous incubation ($p<0.05$). The IC10 values after 1 h or after continuous cisplatin incubation for both cell lines are shown in Table I.

Hyperthermia sensitivity. The sensitivity of both cell lines for hyperthermia treatment at 41 and 43˚C is shown in Fig. 2. Treatment with 43˚C resulted in decreased survival in both cell lines. Although SiHa cells were more sensitive to 43˚C than SW-1573 cells, they were markedly more resistant to 41˚C treatment. While a 4-h treatment at 41˚C decreased the

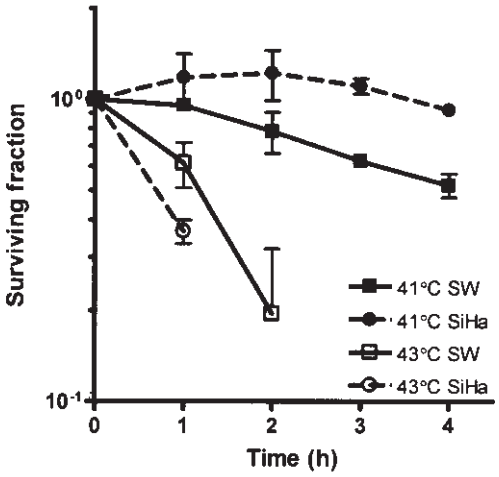

Figure 2. Survival of SW-1573 and SiHa cells after hyperthermia at 41˚C and at 43˚C for exposure times up to 4 h. Means ± SEM are shown for at least three separate experiments.

Table II. Inhibiting exposure time (IET50) after hyperthermia treatment.

	IET50 (min)	
	41˚C (SEM)	43˚C (SEM)
SW-1573	262 (35)	72 (9.2)
SiHa	Not cytotoxic	43 (2.6)

survival of SW-1573 cells to 54±8%, the same treatment did not lead to a significant change in survival of SiHa cells. The IET50 values after hyperthermia at 41˚C and at 43˚C for both cell lines are shown in Table II.

Effect of hyperthermia on cisplatin toxicity. In Fig. 3 it is shown that addition of hyperthermia to cisplatin treatment led to an enhanced cisplatin effect in SW-1573 (Fig. 3a and b) and SiHa (Fig. 3c and d) cells. This effect was achieved after combining 41 or 43˚C hyperthermia for 1 h with simultaneous (1 h) cisplatin exposure and with continuous cisplatin exposure. Thermal enhancement of 1-h cisplatin cytotoxicity was significant ($p<0.05$) in SW-1573 for both temperatures. In SiHa cells enhancement at the 10% survival level was only significant for 43˚C hyperthermia. Continuous cisplatin exposure was highly cytotoxic especially in the cisplatin-sensitive SiHa cells. After continuous cisplatin incubation the thermal enhancement of the cisplatin effect was less than after 1-h incubation. The only significant ($p<0.05$) enhancement of cytotoxicity by continuous cisplatin incubation was observed after combination with hyperthermia at 43˚C in SW-1573 cells. Thermal enhancement ratios (TERs) at 10% survival after hyperthermia at 41 or at 43˚C combined with cisplatin are shown in Table III.

Radiation enhancement. The radiation dose survival curves obtained after 41 and 43˚C (only SW-1573 cells) hyperthermia and/or continuous cisplatin incubation are shown in

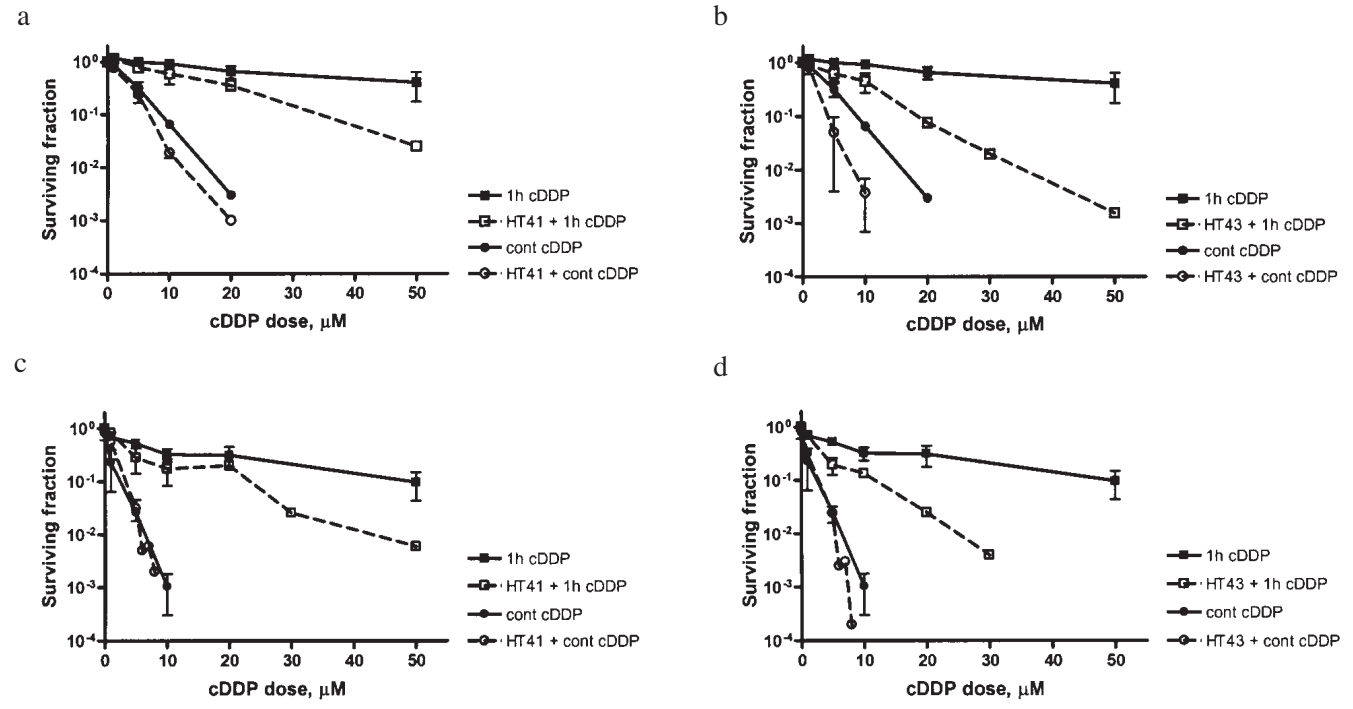

Figure 3. Survival after combined hyperthermia and cisplatin. (a) Survival after 41˚C hyperthermia and cisplatin in SW-1573 cells. (b) Survival after 43˚C hyperthermia and cisplatin in SW-1573 cells. (c) Survival after 41˚C hyperthermia and cisplatin in SiHa cells. (d) Survival after 43˚C hyperthermia and cisplatin in SiHa cells. Mean ± SEM are shown for at least three separate experiments.

Table III. Thermal enhancement ratios (TERs) at 10% survival after hyperthermia combined with cisplatin (cDDP).

	41˚C + 1 h cDDP (SEM)	41˚C + continuous cDDP (SEM)	43˚C + 1 h cDDP (SEM)	43˚C + continuous cDDP (SEM)
SW-1573	3.2 (0.9)[a]	1.1 (0.3)	3.6 (0.6)[a]	2.5 (1.1)[a]
SiHa	2.1 (0.4)	1.6 (0.7)	3.5 (0.1)[a]	0.8 (0.1)

[a]Significant enhancement of TER ($p<0.05$).

Fig. 4. The curves were corrected for the effects of hyperthermia and cisplatin alone and for the combined effect of hyperthermia and cisplatin on cytotoxicity to show the effect on radiosensitivity. The effects of cisplatin and hyperthermia alone on cell survival are shown in Figs. 1 and 2, respectively. The radiation enhancement ratios (RERs) at the 10% survival level after continuous cisplatin, hyperthermia and combined treatment are shown in Table IV.

When cells were pre-treated with only hyperthermia, the results showed that 41˚C hyperthermia did not have an effect on radiosensitivity (Fig. 4a and b; Table IV) whereas 43˚C hyperthermia led to a dramatic increase ($p<0.001$) in radiosensitivity in both cell lines (Fig. 4c for SW-1573 cells; Table IV). Cisplatin treatment (1, 5, and 10 μM for SiHa cells; and 1, 5, 10, and 16 μM for SW-1573 cells) for 1 h did not affect radiosensitivity in both cell lines (data not shown) whereas continuous cisplatin incubation (0.35 and 1 μM for SiHa cells; and 1, 3.55, and 5 μM for SW-1573 cells) had a small radiosensitizing effect in both cell lines (Fig. 4a, b and c). The enhancement in radiosensitivity by 1-μM continuous cisplatin treatment as shown in Fig. 4a and b was significant in SW-1573 cells ($p<0.001$). For SiHa cells data on 5 μM of cisplatin could not be obtained because the treatment was too toxic. Treatment with increasing concentrations of cisplatin led to a small decrease in radiosensitivity in both cell lines. This was observed only at high radiation doses both after 1 h and after continuous cisplatin incubation. This decrease in radiosensitivity is not observed in Table IV, which shows radiation enhancement at the 10% survival level (low radiation doses). Combined treatment with 41˚C hyperthermia and 1 and 5 μM of continuous cisplatin did not enhance radiosensitivity in SW-1573 cells compared with cisplatin alone (Fig. 4a for 1 μM cisplatin; Table IV). In SiHa cells, on the other hand, the same treatment resulted in increased radiosensitivity ($p<0.001$) compared with cisplatin alone (Fig. 4b for 1 μM cisplatin; Table IV). Combined 43˚C hyperthermia and 5 μM of continuous cisplatin incubation led to a significant increase in radiosensitivity ($p<0.001$) of SW-1573 cells (Fig. 4c; Table IV) compared with 5 μM of continuous cisplatin alone.

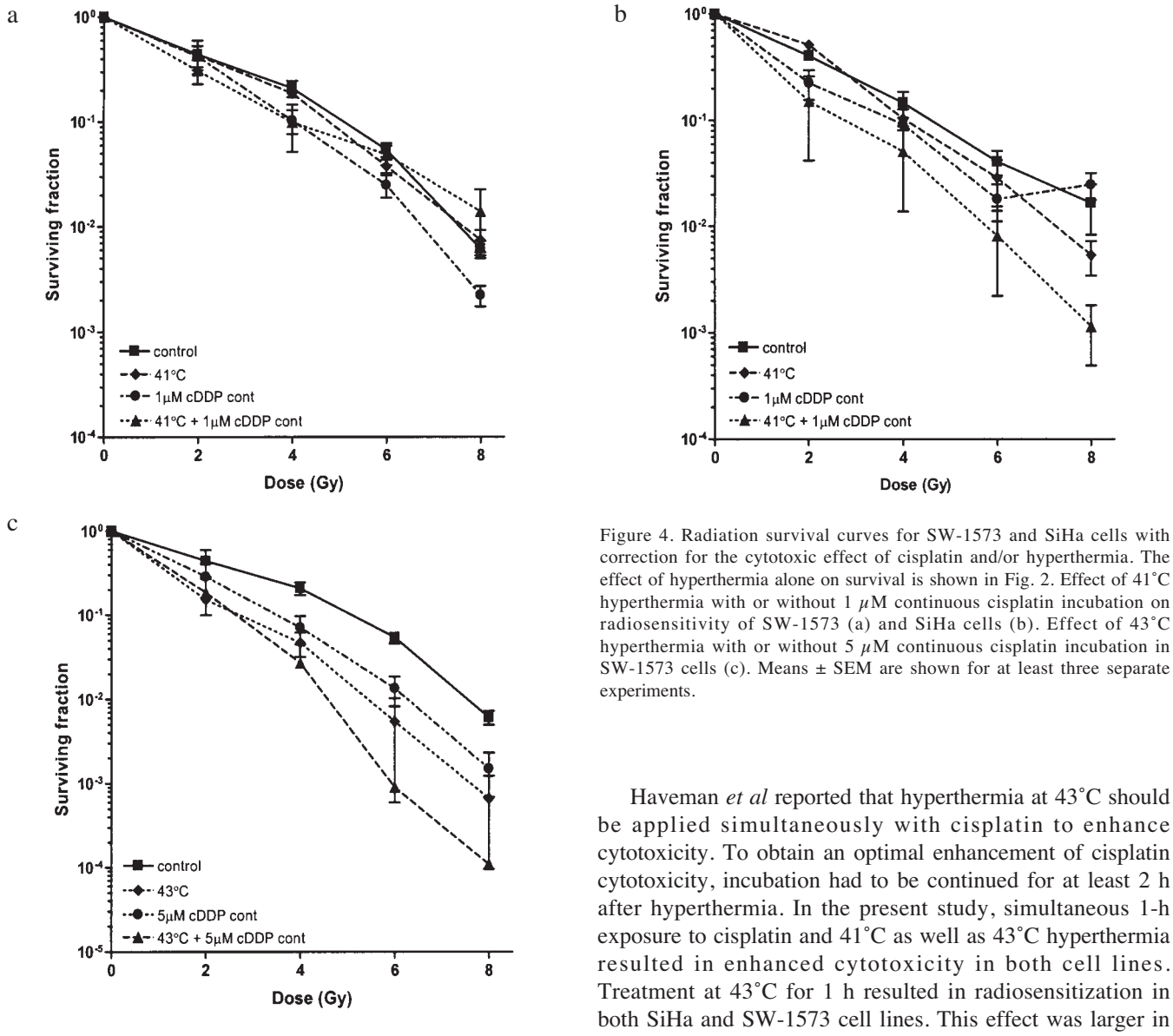

Figure 4. Radiation survival curves for SW-1573 and SiHa cells with correction for the cytotoxic effect of cisplatin and/or hyperthermia. The effect of hyperthermia alone on survival is shown in Fig. 2. Effect of 41˚C hyperthermia with or without 1 μM continuous cisplatin incubation on radiosensitivity of SW-1573 (a) and SiHa cells (b). Effect of 43˚C hyperthermia with or without 5 μM continuous cisplatin incubation in SW-1573 cells (c). Means ± SEM are shown for at least three separate experiments.

Discussion

Our results show that trimodality treatment with hyperthermia, cisplatin and radiation leads to additional tumour cell kill compared with combined radiation and cisplatin. For the cisplatin-sensitive SiHa cell line this was achieved even at a mild temperature of 41˚C, which is not cytotoxic by itself.

Haveman *et al* reported that hyperthermia at 43˚C should be applied simultaneously with cisplatin to enhance cytotoxicity. To obtain an optimal enhancement of cisplatin cytotoxicity, incubation had to be continued for at least 2 h after hyperthermia. In the present study, simultaneous 1-h exposure to cisplatin and 41˚C as well as 43˚C hyperthermia resulted in enhanced cytotoxicity in both cell lines. Treatment at 43˚C for 1 h resulted in radiosensitization in both SiHa and SW-1573 cell lines. This effect was larger in SiHa cells than in SW-1573 cells. These results are in agreement with those of many other studies on hyperthermia treatment at temperatures >42˚C which also found enhancement of radiosensitivity (4,5,7,33,34). At higher temperatures, radiosensitization is thought to occur primarily via DNA repair inhibition (1). In contrast, hyperthermia treatment at 41˚C did not result in radiosensitization of both SiHa and SW-1573 cells. Results from other *in vitro* studies on mild hyperthermia vary from no effect on radiosensitivity to a

Table IV. Radiation enhancement ratios (RERs) at 10% survival after cisplatin (cDDP), hyperthermia or combined cisplatin and hyperthermia treatment.

	Continuous cDDP (SEM)		41˚C (SEM)	41˚C + continuous 1 μM cDDP (SEM)	43˚C (SEM)	43˚C + continuous 5 μM cDDP (SEM)
	1 μM	5 μM				
SW-1573	1.4 (0.2)[a]	1.5 (0.2)[a]	1.0 (0.1)	1.1 (0.2)	1.7 (0.1)[a]	2.1 (0.3)[b]
SiHa	1.0 (0.3)	Too cytotoxic	1.3 (0.3)	2.0 (1.1)[b]	2.2 (0.2)[a]	Too cytotoxic

[a]Significant ($p<0.001$) radiation enhancement after combination treatments compared with radiation alone; [b]significant ($p<0.001$) radiation enhancement after trimodality treatment compared with radiation and continuous 1-μM (SiHa) or 5-μM (SW-1573) treatment.

clear hyperthermia-induced radiosensitization (33,35-37). As mentioned earlier, an important mechanism of mild hyperthermia-induced radiosensitization *in vivo* is the reoxygenation of tumours by an increase in blood flow (38,39).

Cisplatin has been shown to induce radiosensitization (23,24,40). In the present study, cisplatin only induced radiosensitization in both cell lines when the incubation was prolonged after irradiation. Cisplatin-induced radiosensitization has been shown to occur via inhibition of the non-homologous end joining (NHEJ) pathway (41-43). We observed that the extent of radiosensitization decreased with increasing radiation and cisplatin doses. Other authors have suggested that this might be due to a small cisplatin-resistant subpopulation remaining after treatment with high concentrations of cisplatin (44,45).

Cisplatin resistance can both be natural or acquired after initial treatment and genetic selection (46). The phenomenon is multifactorial and has been the subject of many studies. In general, it consists of mechanisms limiting the formation of DNA adducts, enhanced repair of adducts and/or increased tolerance of the resulting DNA damage (46). A decreased intracellular cisplatin accumulation has been found in cisplatin-resistant cells (21,47). The nature of the difference in cisplatin sensitivity of the cell lines used in the present study is unknown. It may involve a combination of differences in cisplatin transport mechanisms, detoxification mechanisms, DNA repair, chromatin structure and/or signal transduction pathways (48). Cisplatin resistance has been shown to be (partially) abolished by hyperthermia because it increases the amount of cisplatin-DNA adducts (49-52). A study by Raaphorst *et al* comparing human ovarian carcinoma and glioma cell lines sensitive and resistant to cisplatin shows that the degree of sensitization to cisplatin by hyperthermia is dependent on the cell line and on the temperature applied (53). In this study, both cell lines had a cisplatin-resistant variant. In the ovarian carcinoma system, hyperthermia at temperatures ranging from 41-45˚C administered concurrently with cisplatin for 1 h had a slightly greater cisplatin-sensitizing effect on the resistant cell line whereas in the glioma system the opposite was true. However, significant thermal sensitization was achieved in all cell lines at temperatures ranging from 41-45˚C (53). We observed a greater cisplatin sensitization by hyperthermia at 41˚C and at 43˚C administered concurrently with 1-h cisplatin treatment in the cisplatin-sensitive SiHa cell line than in the resistant SW-1573 cells. When cisplatin incubation was continuous, thermal enhancement was greater in SW-1573 cells. Perhaps the continuous incubation had already led to such a high cellular cisplatin accumulation in the sensitive SiHa cells that hyperthermia could not elevate cisplatin levels in these cells.

We proposed that trimodality treatment consisting of radiation, cisplatin and hyperthermia may improve treatment outcome by increasing the cellular radiosensitivity compared with cisplatin alone. In SW-1573 cells the addition of hyperthermia at a clinically relevant temperature of 41˚C did not further enhance radiosensitivity when combined with cisplatin. Trimodality treatment using hyperthermia at 43˚C, on the other hand, led to increased sensitivity compared to cisplatin and radiation. Apparently, the remaining cisplatin-resistant subpopulations as mentioned earlier (44,45) are also resistant to 41˚C hyperthermia treatment, and a higher temperature is necessary in order to eradicate these cells. In SiHa cells it was not possible to study the effect of increasing cisplatin concentration because continuous incubation with cisplatin concentrations >1 μM was too cytotoxic. However, in this cell line, combined treatment with 41˚C hyperthermia and cisplatin was able to increase sensitivity compared with cisplatin and radiation. Other studies on trimodality treatment with cisplatin, 40˚C hyperthermia and radiation showed a synergistic interaction between the modalities both in cisplatin-sensitive and cisplatin-resistant cell lines (25,26,54,55). However, these studies focused on long-duration hyperthermia, low dose-rate irradiation (LDRI) or split-dose irradiation. Another study on trimodality treatment using hyperthermia at 44˚C for 30 min in a human glioblastoma and a canine glioma cell line observed only an additive effect (27).

In conclusion, combined cisplatin and 41˚C hyperthermia treatment increased the radiosensitivity of SiHa cells. In SW-1573, in contrast, the combination of 41˚C with cisplatin did not increase radiosensitivity. However, an enhancement of radiosensitivity was obtained using 43˚C treatment. The implication of the present study is that tumours that are more resistant to cisplatin may not respond to low-temperature hyperthermia. Therefore, the goal must be to keep tumour temperatures as high as possible. In this way, trimodality treatment can be beneficial to patients by increasing cellular radiosensitivity in both cisplatin-sensitive and -resistant tumours. The first results from a clinical study on trimodality treatment in advanced stage cervical carcinoma patients are very encouraging. They show that the treatment is feasible and effective and is well tolerated by patients (28).

Acknowledgements

The authors wish to thank Dr H. Crezee for critically reviewing the manuscript. The Maurits and Anna de Kock foundation is acknowledged for sponsoring laboratory equipment.

References

1. Kampinga HH and Dikomey E: Hyperthermic radiosensitization: mode of action and clinical relevance. Int J Radiat Biol 77: 399-408, 2001.
2. Roti Roti JL: Introduction: radiosensitization by hyperthermia. Int J Hyperthermia 20: 109-114, 2004.
3. Hildebrandt B, Wust P, Ahlers O, *et al*: The cellular and molecular basis of hyperthermia. Crit Rev Oncol Hematol 43: 33-56, 2002.
4. Woudstra EC, Konings AW, Jeggo PA and Kampinga HH: Role of DNA-PK subunits in radiosensitization by hyperthermia. Radiat Res 152: 214-218, 1999.
5. Dynlacht JR, Bittner ME, Bethel JA and Beck BD: The non-homologous end-joining pathway is not involved in the radiosensitization of mammalian cells by heat shock. J Cell Physiol 196: 557-564, 2003.
6. Warters RL and Axtell J: Repair of DNA strand breaks at hyperthermic temperatures in Chinese hamster ovary cells. Int J Radiat Biol 61: 43-48, 1992.
7. Dewey WC, Sapareto SA and Betten DA: Hyperthermic radiosensitization of synchronous Chinese hamster cells: relationship between lethality and chromosomal aberrations. Radiat Res 76: 48-59, 1978.
8. Raaphorst GP, Yang DP and Niedbala G: Is DNA polymerase beta important in thermal radiosensitization? Int J Hyperthermia 20: 140-143, 2004.

9. Kampinga HH, Hiemstra YS, Konings AW and Dikomey E: Correlation between slowly repairable double-strand breaks and thermal radiosensitization in the human HeLa S3 cell line. Int J Radiat Biol 72: 293-301, 1997.
10. Ben Hur E, Elkind MM and Bronk BV: Thermally enhanced radioresponse of cultured Chinese hamster cells: inhibition of repair of sublethal damage and enhancement of lethal damage. Radiat Res 58: 38-51, 1974.
11. Kampinga HH, Dynlacht JR and Dikomey E: Mechanism of radiosensitization by hyperthermia (> or = 43 degrees C) as derived from studies with DNA repair defective mutant cell lines. Int J Hyperthermia 20: 131-139, 2004.
12. Kampinga HH, Kanon B, Konings AW, Stackhouse MA and Bedford JS: Thermal radiosensitization in heat- and radiation-sensitive mutants of CHO cells. Int J Radiat Biol 64: 225-230, 1993.
13. Laszlo A, Davidson T, Harvey A, *et al*: Alterations in heat-induced radiosensitization accompanied by nuclear structure alterations in Chinese hamster cells. Int J Hyperthermia 22: 43-60, 2006.
14. Kampinga HH: Cell biological effects of hyperthermia alone or combined with radiation or drugs: a short introduction to newcomers in the field. Int J Hyperthermia 22: 191-196, 2006.
15. Haveman J, Bergs JW, Franken NA, van Bree C and Stalpers LJ: Effect of hyperthermia on uptake and cytotoxicity of cisplatin in cultured murine mammary carcinoma cells. Oncol Rep 14: 561-567, 2005.
16. van Bree C, Rietbroek RC, Schopman EM, Kipp JB and Bakker PJ: Local hyperthermia enhances the effect of cis-diamminedichloroplatinum(II) on nonirradiated and preirradiated rat solid tumors. Int J Radiat Oncol Biol Phys 36: 135-140, 1996.
17. Wondergem J, Bulger RE, Strebel FR, *et al*: Effect of cis-diamminedichloroplatinum(II) combined with whole body hyperthermia on renal injury. Cancer Res 48: 440-446, 1988.
18. Los G, van Vugt MJ and Pinedo HM: Response of peritoneal solid tumours after intraperitoneal chemohyperthermia treatment with cisplatin or carboplatin. Br J Cancer 69: 235-241, 1994.
19. Urano M, Kahn J, Majima H and Gerweck LE: The cytotoxic effect of cis-diamminedichloroplatinum(II) on cultured Chinese hamster ovary cells at elevated temperatures: Arrhenius plot analysis. Int J Hyperthermia 6: 581-590, 1990.
20. Herman TS, Teicher BA, Cathcart KN, Kaufmann ME, Lee JB and Lee MH: Effect of hyperthermia on cis-diamminedichloroplatinum(II) (rhodamine 123)2[tetrachloroplatinum(II)] in a human squamous cell carcinoma line and a cis-diamminedichloroplatinum(II)-resistant subline. Cancer Res 48: 5101-5105, 1988.
21. Wallner KE, DeGregorio MW and Li GC: Hyperthermic potentiation of cis-diamminedichloroplatinum(II) cytotoxicity in Chinese hamster ovary cells resistant to the drug. Cancer Res 46: 6242-6245, 1986.
22. Ohtsubo T, Saito H, Tanaka N, Tsuzuki H, Saito T and Kano E: *In vitro* effect of hyperthermia on chemoenhancement and uptake of cisplatin in human pharyngeal carcinoma KB cells. Chemotherapy 43: 43-50, 1997.
23. Wilkins DE, Heller DP and Raaphorst GP: Inhibition of potentially lethal damage recovery by cisplatin in a brain tumor cell line. Anticancer Res 13: 2137-2142, 1993.
24. Bergs JW, Franken NA, ten Cate R, van Bree C and Haveman J: Effects of cisplatin and gamma-irradiation on cell survival, the induction of chromosomal aberrations and apoptosis in SW-1573 cells. Mutat Res 594: 148-154, 2006.
25. Raaphorst GP, Maio J, Ng CE and Stewart DJ: Concomitant treatment with mild hyperthermia, cisplatin and low dose-rate irradiation in human ovarian cancer cells sensitive and resistant to cisplatin. Oncol Rep 5: 971-977, 1998.
26. Raaphorst GP, Yang H, Wilkins DE and Ng CE: Cisplatin, hyperthermia and radiation treatment in human cisplatin-sensitive and -resistant glioma cell lines. Int J Hyperthermia 12: 801-812, 1996.
27. Salcman M and Ebert PS: *In vitro* response of human glioblastoma and canine glioma cells to hyperthermia, radiation, and chemotherapy. Neurosurgery 29: 526-531, 1991.
28. Westermann AM, Jones EL, Schem BC, *et al*: First results of triple-modality treatment combining radiotherapy, chemotherapy, and hyperthermia for the treatment of patients with stage IIB, III, and IVA cervical carcinoma. Cancer 104: 763-770, 2005.
29. Dewhirst MW, Vujaskovic Z, Jones E and Thrall D: Re-setting the biologic rationale for thermal therapy. Int J Hyperthermia 21: 779-790, 2005.
30. van der Zee J, Gonzalez GD, van Rhoon GC, van Dijk JD, van Putten WL and Hart AA: Comparison of radiotherapy alone with radiotherapy plus hyperthermia in locally advanced pelvic tumours: a prospective, randomised, multicentre trial. Dutch Deep Hyperthermia Group. Lancet 355: 1119-1125, 2000.
31. Franken NAP, Rodermond HM, Stap J, Haveman J and van Bree C: The clonogenic assay of cells *in vitro*. Nat Protocols (In press).
32. Nagai N, Kinoshita M, Ogata H, *et al*: Relationship between pharmacokinetics of unchanged cisplatin and nephrotoxicity after intravenous infusions of cisplatin to cancer patients. Cancer Chemother Pharmacol 39: 131-137, 1996.
33. Raaphorst GP, Heller DP, Bussey A and Ng CE: Thermal radiosensitization by 41 degrees C hyperthermia during low dose-rate irradiation in human normal and tumour cell lines. Int J Hyperthermia 10: 263-270, 1994.
34. El Awady RA, Dikomey E and Dahm-Daphi J: Heat effects on DNA repair after ionising radiation: hyperthermia commonly increases the number of non-repaired double-strand breaks and structural rearrangements. Nucleic Acids Res 29: 1960-1966, 2001.
35. Xu M, Wright WD, Higashikubo R, Wang LL and Roti Roti JL: Thermal radiosensitization of human tumour cell lines with different sensitivities to 41.1 degrees C. Int J Hyperthermia 15: 279-290, 1999.
36. Ryu S, Brown SL, Kim SH, Khil MS and Kim JH: Preferential radiosensitization of human prostatic carcinoma cells by mild hyperthermia. Int J Radiat Oncol Biol Phys 34: 133-138, 1996.
37. Myerson RJ, Roti Roti JL, Moros EG, Straube WL and Xu M: Modelling heat-induced radiosensitization: clinical implications. Int J Hyperthermia 20: 201-212, 2004.
38. Oleson JR: Eugene Robertson special lecture. Hyperthermia from the clinic to the laboratory: a hypothesis. Int J Hyperthermia 11: 315-322, 1995.
39. Song CW, Shakil A, Osborn JL and Iwata K: Tumour oxygenation is increased by hyperthermia at mild temperatures. Int J Hyperthermia 12: 367-373, 1996.
40. Begg AC, van der Kolk PJ, Dewit L and Bartelink H: Radiosensitization by cisplatin of RIF1 tumour cells *in vitro*. Int J Radiat Biol Relat Stud Phys Chem Med 50: 871-884, 1986.
41. Diggle CP, Bentley J, Knowles MA and Kiltie AE: Inhibition of double-strand break non-homologous end-joining by cisplatin adducts in human cell extracts. Nucleic Acids Res 33: 2531-2539, 2005.
42. Wilson GD, Bentzen SM and Harari PM: Biologic basis for combining drugs with radiation. Semin Radiat Oncol 16: 2-9, 2006.
43. Boeckman HJ, Trego KS and Turchi JJ: Cisplatin sensitizes cancer cells to ionizing radiation via inhibition of non-homologous end joining. Mol Cancer Res 3: 277-285, 2005.
44. Gorodetsky R, Levy-Agababa F, Mou X and Vexler AM: Combination of cisplatin and radiation in cell culture: effect of duration of exposure to drug and timing of irradiation. Int J Cancer 75: 635-642, 1998.
45. Myint WK, Ng C and Raaphorst GP: Examining the non-homologous repair process following cisplatin and radiation treatments. Int J Radiat Biol 78: 417-424, 2002.
46. Brabec V and Kasparkova J: Molecular aspects of resistance to antitumor platinum drugs. Drug Resist Updat 5: 147-161, 2002.
47. Mansouri A, Henle KJ, Benson AM, Moss AJ and Nagle WA: Characterization of a cisplatin-resistant subline of murine RIF-1 cells and reversal of drug resistance by hyperthermia. Cancer Res 49: 2674-2678, 1989.
48. Hettinga JVE: Reduction of cisplatin resistance by hyperthermia. Thesis at the University of Groningen, 1996.
49. Meyn RE, Corry PM, Fletcher SE and Demetriades M: Thermal enhancement of DNA damage in mammalian cells treated with cis-diamminedichloroplatinum(II). Cancer Res 40: 1136-1139, 1980.
50. Hettinga JV, Lemstra W, Meijer C, *et al*: Mechanism of hyperthermic potentiation of cisplatin action in cisplatin-sensitive and -resistant tumour cells. Br J Cancer 75: 1735-1743, 1997.
51. Krishnaswamy G and Dewey WC: Cell killing and chromosomal aberrations induced in Chinese hamster ovary cells by treating with cisplatin at 41.5 degrees C during the G1 or late S phase. Cancer Res 53: 1239-1243, 1993.

52. van de Vaart PJ, van der Vange N, Zoetmulder FA, *et al*: Intraperitoneal cisplatin with regional hyperthermia in advanced ovarian cancer: pharmacokinetics and cisplatin-DNA adduct formation in patients and ovarian cancer cell lines. Eur J Cancer 34: 148-154, 1998.
53. Raaphorst GP, Doja S, Davis L, Stewart D and Ng CE: A comparison of hyperthermia cisplatin sensitization in human ovarian carcinoma and glioma cell lines sensitive and resistant to cisplatin treatment. Cancer Chemother Pharmacol 37: 574-580, 1996.
54. Raaphorst GP, Miao J, Stewart D and Ng CE: Interactions of mild hyperthermia, cisplatin and split dose irradiation in human ovarian carcinoma cells. Cancer Chemother Pharmacol 41: 491-496, 1998.
55. Raaphorst GP, Miao J and Ng CE: Cisplatin and mild hyperthermia in radiosensitization to low dose rate irradiation in human ovarian carcinoma cells. Anticancer Res 17: 3469-3472, 1997.