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● *Biology Contribution*

HYPERTHERMIA AND INCORPORATION OF HALOGENATED PYRIMIDINES: RADIOSENSITIZATION IN CULTURED RODENT AND HUMAN TUMOR CELLS

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Purpose: To investigate the possible benefit of hyperthermia (HT) in combination with radiosensitization by halogenated pyrimidines (HPs) in rodent as well as in human tumor cells.

Methods and Materials: Exponentially growing rodent cells, radiosensitive R-1 and MOS cells and radioresistant RUC-II and V79 cells, and human SW1573 cells, were exposed to 0, 1, 2, and 4 μ M of chloro- (CldUrd), bromo- (BrdUrd), or iodo-deoxyuridine (IdUrd) in the culture medium. Survival after irradiation with gamma-rays from a ¹³⁷Cs source and/or hyperthermic treatment (HT, 60 min at 42°C) was determined by clonogenic assay. Linear-quadratic analyses of the radiation survival curves were performed to assess sensitization in the dose range 1 to 3 Gy relevant to radiotherapy.

Results: The incorporation of HPs sensitized all cell lines to HT and resulted in radiosensitization dependent on the percentage of thymidine replacement. At equal levels of thymidine replacement, IdUrd was the most potent radiosensitizer. HT further increased radiation-induced lethality of cells that had incorporated HPs. Linear-quadratic analyses showed that HT further increased the linear parameter of the LQ formula while the quadratic parameter was not significantly changed.

Conclusion: The combination of HT and HPs act additively in increasing the radiosensitivity of rodent tumor cell lines with varying radiosensitivities as well as of a human tumor cell line. In particular, the ratio of the linear parameter to the quadratic parameter, relevant for fractionation effects in radiotherapy, was increased. © 1997 Elsevier Science Inc.

Hyperthermia, Halogenated pyrimidines, Radiation, Cell culture, LQ analysis.

INTRODUCTION

The incorporation of halogenated pyrimidines (HPs) is known to increase the radiosensitivity of mammalian cells *in vitro* and *in vivo*. The HPs bromo-deoxyuridine (BrdUrd) and iodo-deoxyuridine (IdUrd) are already applied clinically to enhance loco-regional effectiveness (8, 28, 31, 35). The level of radiosensitization by HPs has been shown to correlate with the degree of thymidine replacement (6, 16, 27, 32). However, in recent reports it has been argued that the fraction of labeled cells in tumors may be too low for a significant clinical success of HP-induced radiosensitization, although the level of thymidine replacement by HPs in proliferating tumor cells would allow more sensitization (7, 30). The optimal administration schedule for HPs is still a matter of debate (7, 16, 35).

Hyperthermia (HT) is also known to sensitize cells to radiation and has recently been shown to be beneficial in

combination with radiotherapy (9, 25). It has been reported that HT can block the repair of DNA double-strand breaks (23), which are supposed to be the lesions by which radiation kills cells (26). The incorporation of HPs have been shown to increase the amount of radiation-induced DNA double-strand breaks (14, 38, 39).

The influence of modifying agents on radiation survival curves of mammalian cells is analyzed increasingly in terms of changes in the parameters derived from the description of the shapes of these curves according to the linear-quadratic model (LQ) (17, 20–22). This model has been found to describe the low-dose region of the curves better than the single-hit multitarget model (2, 33). The LQ model leads to a description of survival curves by the formula: $S(D)/S(0) = \exp - (\alpha D + \beta D^2)$. Using this model, more insight can be obtained into the quantitative aspects of the sensitization of tumors and their constituent cells by a combination of HT and incorporation

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of HPs, especially in the dose range of 1 to 3 Gy, as commonly applied in fractionated radiotherapy.

Several groups have studied the effect of HPs on radiosensitization in human cancer cell lines of different radiosensitivities (16, 17, 20–22, 34). In studies of the radiosensitization of human colon cancer cell lines by incorporation of the IdUrd and BrdUrd, it has been shown that the linear term in the LQ formula, which dominates the response at low doses, is strongly increased, but that the quadratic term is hardly affected (17, 20–22). In otherwise untreated cells, it has been reported that HT increased the quadratic parameter and to a lesser extent also the linear parameter (10). Previous reports of studies on the radiosensitivity of cultured Chinese hamster cells, in which effects of HT and incorporation of HPs in DNA were measured, did not incorporate an analysis in terms of the LQ model, but changes were evaluated in terms of a final slope and extrapolation number of survival curves (29).

To study the possible improvement of the efficacy of HP-induced radiosensitization, we performed experiments on the effectiveness of radiosensitization by HPs in combination with HT. In the present report, we compare the effects of HPs and HT and study the combination of these treatments for tumor cells *in vitro*. It is generally recognized that the effectiveness of fractionated radiotherapy with dose fractions of 2 Gy is largely determined by the linear parameter of the LQ formula. Because cells with a small linear parameter respond poorly to fractionated radiotherapy, we selected two tumor cell lines of different radiosensitivities that can be analyzed quantitatively *in vitro* and that can be transplanted into rats for future *in vivo* studies (36, 37). We extended the investigations with a murine and a human tumor cell line and with Chinese hamster lung fibroblasts to assess a wide range of linear and quadratic parameters.

MATERIALS AND METHODS

Cell culture

Cultures of cells from a rat R-1 rhabdomyosarcoma, a rat RUC-II urethra carcinoma, a murine MOS osteosarcoma, a human SW1573 lung carcinoma, and Chinese hamster V79 lung fibroblasts were used. Cell cycle times were 10–12 h for RUC-II, MOS, and V79 cells, 14–16 h for R-1 cells, and 20–23 h for SW1573 cells (1, 11, 15). Cells were grown as monolayers in tissue culture flasks¹ (75 cm²) in the appropriate growth medium (rodent cells in minimal essential medium² in an atmosphere of 2% CO₂ in air, human cells in Leibovitz-15 medium² in air, both supplemented with 10% fetal bovine serum, glutamine, and penicillin at 37°C). All cell lines were passaged twice a week at moderate den-

sity ($1-2 \times 10^5$ cells per flask) to maintain exponential growth. For experiments, the cells were plated at low density ($2-5 \times 10^4$ cells per flask) to ensure that the cells were growing exponentially during the entire drug exposure interval. The rodent cells were incubated for 72 h in 10 ml MEMs in the presence of 1, 2, and 4 μ M of chloro-deoxyuridine (CldUrd), BrdUrd, or IdUrd.³ The human cells were incubated for 96 h because of their longer cell cycle time. The highest HP concentrations did not significantly slow down cell proliferation, and flow cytometry studies showed that no cell cycle redistribution occurred (data not shown). In rodent cells, thymidine was added at a concentration of 2.5 μ M to mimic the average level of thymidine in rodent plasma (30).

Incorporation of HPs

Thymidine replacement was measured by the technique described by Miller *et al.* (21). In short, cells were plated at a cell density of $0.5-1 \times 10^5$ per flask and treated with CldUrd, BrdUrd, and IdUrd, as described above. Cells were trypsinized, pelleted, and lysed in water. After isolation from the lysate, DNA was digested to liberate nucleosides. The digest was analyzed by high-pressure liquid chromatography. Percentage thymidine replacement was calculated as the concentration of incorporated HP $\times 100$ divided by the sum of the concentrations thymidine and incorporated HP. Recovery differences between samples were monitored by comparison of the concentration of guanine divided by the sum of the concentrations of thymidine and incorporated HP.

Irradiation and hyperthermia

For the assessment of radiation survival after exposure to CldUrd, BrdUrd, or IdUrd, the cells were trypsinized and plated in appropriate dilutions in growth medium in six-well macroplates.⁴ Four hours after plating, the cells were irradiated with gamma-rays from a ¹³⁷Cs source, yielding a dose rate of about 1 Gy/min. For HT, the macroplates were placed immediately after irradiation in a thermostatically regulated waterbath for 60 min at 42.0°C.

Analysis and statistics

Following irradiation and/or HT, the cells were incubated for 5–7 days without changing the culture medium. Subsequently, the colonies were fixated with 100% ethanol and stained with 10% Giemsa. Colonies of 50 cells or more were scored as originating from a single clonogenic cell. Surviving fractions were calculated and analyses were performed by fitting the survival curves according to the LQ formula using statistical software⁵ performing a fit to the data by multiple regression. Statistical analyses were performed with statistical software using the two-sided Student's *t*-test.

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⁵ BMDP Statistical Software, Los Angeles, CA.

Fig. 1. Radiation dose-survival curves of rodent R-1, MOS, RUC-II, and V79 cell lines with varying radiosensitivity and of the human SW1573 tumor cell line. Mean values with standard errors of the mean of at least three separate experiments are plotted. Lines represent polynomial fits to the data.

RESULTS

Radiation dose-survival curves of the radiosensitive R-1 and MOS cell lines and the more radioresistant RUC-II and V79 cell lines and the human SW1573 cell line are shown in Fig. 1. The linear term determining the initial slope (α -parameter) and the quadratic term determining the continuously curving high dose region (β -parameter) of the radiation survival curves of these cell lines are presented in Table 1 and are shown to differ considerably.

The exposure to HPs at the highest concentrations induced a significant reduction in the clonogenic capacity compared to the control. Most toxicity was observed with BrdUrd in the more radiosensitive cell lines (up to 90% cell kill, data not shown). Surviving fractions after irradiation were always corrected for any decrease in plating efficiency.

As shown in Fig. 2, the exposure to the highest concentration of CldUrd and BrdUrd resulted in a significant reduction in the surviving fraction after HT alone in all cell lines. For IdUrd, a significantly enhanced thermal sensitivity was observed in the RUC-II, MOS, and SW1573 cell lines.

Examples of radiation dose-survival curves of R-1 and RUC-II cells after exposure to the highest HP concentration with and without HT are shown in Fig. 3. The exposure of HPs resulted in radiosensitization that depends on HP concentration in the medium (data not shown).

Table 1. Linear and quadratic parameters* of control radiation dose-survival curves

Cell line	α , Gy ⁻¹	β , Gy ⁻²	α/β , Gy
R-1	0.23 $\pm$ 0.01	0.068 $\pm$ 0.003	3.4
RUC-II	0.008 $\pm$ 0.007	0.025 $\pm$ 0.001	0.3
MOS	0.16 $\pm$ 0.05	0.111 $\pm$ 0.001	1.4
V79	0.15 $\pm$ 0.03	0.013 $\pm$ 0.003	11.5
SW1573	0.26 $\pm$ 0.03	0.019 $\pm$ 0.004	13.7

* Means with standard errors of the mean of at least three separate experiments.

Treatment with HT of irradiated cells, which had incorporated HPs, resulted in a further decrease in surviving fraction in all cell lines, independent of the HP used.

Linear-quadratic analysis of the radiation dose-survival curves showed that the treatment with HT and HPs significantly increased the α -parameter of all cell lines (see Table 2 for the data on R-1 and RUC-II cells). In Fig. 4, the effects of HT and incorporation of HPs on the linear parameter of the LQ formalism of R-1 and RUC-II cells are shown as a function of thymidine replacement. Linear correlations were derived between the α -parameter and thymidine replacement. At equal levels of thymidine replacement, IdUrd was found to be the most potent radiosensitizer, followed by BrdUrd and CldUrd. It could also be deduced that the α -value of the radioresistant RUC-II cells was increased by multiplication factors in excess of 50. Furthermore, it was observed that HT and

Fig. 2. Survival of the examined cell lines after treatment with hyperthermia (60 min at 42°C) after exposure to 4 μ M CldUrd, BrdUrd, or IdUrd. Mean values with standard errors of the mean of at least three separate experiments are plotted. Asterisks indicate significant differences from controls (* p < 0.05; ** p < 0.01, *** p < 0.001).

Fig. 3. Radiation dose-survival curves showing radiosensitization of radiosensitive R-1 (A) and radioresistant RUC-II (B) cells after exposure to 0 μ M, 4 μ M CldUrd, BrdUrd, or IdUrd

HPs did not change the β -parameter by as large a factor. As a consequence, the α/β ratio for all cell lines increased (see Table 2 for the data on R-1 and RUC-II cells).

An overview of the changes in the α -parameter induced by the most potent radiosensitizer, IdUrd, and HT is shown in Fig. 5. The combination of IdUrd and HT resulted in a marked increase in all cell lines. The relative increase in the linear parameter, i.e., values without HP incorporation, were taken as unity, indicated that HPs caused an increase in the range of 2–5 at the highest thymidine replacement, while HT alone increased the α -parameter two- to fourfold (data not shown). Comparison of the results on the relative increase in the linear parameter after treatment with the combination of IdUrd and HT indicated that the values obtained after single treatments were approximately additive.

DISCUSSION

The *in vitro* experiments reported in this study were aimed at investigating the possible influence of HT in combination with radiosensitization by HPs. In our initial experiments, we measured HP-induced radiosensitization to compare with published data. Our data confirm that the level of radiosensitization by incorporation of CldUrd, BrdUrd, and IdUrd is directly correlated with the degree of thymidine replacement (6, 16, 27, 32).

In our studies, it was also found that the incorporation of CldUrd and BrdUrd plateaued at 30 to 35% of thymidine replacement (16). The incorporation of IdUrd into DNA was lower in all cell lines. It has been reported that human colon cancer cells in a murine xenograft model incorporated less IdUrd as expected from *in vitro* data (30). Because IdUrd incorporation is probably lowered by endogenous plasma thymidine concentration, which in rodents is much higher than in humans (24), thymidine was added to the culture medium of rodent cell lines to mimic the plasma thymidine levels. On the other hand, our data demonstrate that without additional thymidine IdUrd incorporation in SW1573 cells was significantly lower than CldUrd and BrdUrd (data not shown).

Our results furthermore confirm that IdUrd is the most potent radiosensitizer, related to the larger size of I in comparison with Br and Cl (5, 21, 22). Although higher thymidine replacement would result in more radiosensitization, the incorporation of IdUrd in the cell lines studied was similar to the levels presently achieved in the clinic. The observed thymidine replacement already showed significant radiosensitization. Because IdUrd is also preferred over BrdUrd in the clinic because of its reduced toxicity

with or without hyperthermia (60 min at 42°C). Mean values with standard error of the mean of at least three separate experiments are plotted. Lines represent polynomial fits to the data.

Table 2. Effect of HT and incorporation of halogenated pyrimidines on the linear and quadratic parameters* of radiation dose-survival curves of R-1 and RUC-II cells

Cell line	Treatment	α , Gy ⁻¹	β , Gy ⁻²	α/β , Gy
R-1	0 μ M	0.23 $\pm$ 0.01	0.068 $\pm$ 0.003	3.4
	HT	0.66 $\pm$ 0.10	0.061 $\pm$ 0.035	10.8
	4 μ M CldUrd	0.79 $\pm$ 0.10	0.050 $\pm$ 0.030	15.8
	4 μ M CldUrd+HT	1.63 $\pm$ 0.10	n.e.	n.e.
	4 μ M BrdUrd	0.97 $\pm$ 0.12	n.e.	n.e.
	4 μ M BrdUrd+HT	1.49 $\pm$ 0.45	n.e.	n.e.
	4 μ M IdUrd	0.44 $\pm$ 0.05	0.075 $\pm$ 0.016	5.9
	4 μ M IdUrd+HT	0.96 $\pm$ 0.05	0.053 $\pm$ 0.048	18.1
RUC-II	0 μ M	0.008 $\pm$ 0.007	0.025 $\pm$ 0.001	0.3
	HT	0.19 $\pm$ 0.02	0.050 $\pm$ 0.004	3.8
	4 μ M CldUrd	0.09 $\pm$ 0.02	0.037 $\pm$ 0.003	2.4
	4 μ M CldUrd+HT	0.40 $\pm$ 0.05	0.107 $\pm$ 0.017	3.7
	4 μ M BrdUrd	0.15 $\pm$ 0.02	0.044 $\pm$ 0.004	3.4
	4 μ M BrdUrd+HT	0.65 $\pm$ 0.08	0.043 $\pm$ 0.028	15.1
	4 μ M IdUrd	0.06 $\pm$ 0.02	0.026 $\pm$ 0.001	2.3
	4 μ M IdUrd+HT	0.33 $\pm$ 0.05	0.040 $\pm$ 0.010	8.3

* Means with standard errors of the mean of at least three separate experiments; HT is hyperthermia 60 min at 42°C. n.e. = not evaluable because of the unreliable calculation of the β -parameter due to the large value of α .

(8), we have focussed our studies on IdUrd in combination with HT.

Our linear-quadratic analyses clearly demonstrate that HP-induced radiosensitization in exponentially growing cells is dominated by an increase in the linear parameter, but that the quadratic parameter is much less affected (17, 20–22). It has been reported that radioresistant cells can be sensitized to a larger extent by HP incorporation than radiosensitive cells (21, 22, 34). In our study, the degree of radiosensitization of radiosensitive R-1, MOS, and SW1573 cells was similar to that of the rather radioresistant V79 cells. However, as shown in Table 1, the apparent radioresistance of V79 cells is represented by a very small β -parameter. Because the effect of HP-induced radiosensitization in exponentially growing cells is mainly on the α -parameter, no differences could be expected between V79 cells and the radiosensitive cell lines studied. Only for the extremely radioresistant RUC-II cells larger multiplication factors were obtained, probably related to the very low α -value of the control curve. In earlier studies, it has been demonstrated that the radioresistant RUC-II cells showed relatively more sensitivity to higher LET irradiation compared to the radiosensitive R-1 cells (3). This is in agreement with the hypothesis that HP-induced radiosensitization is caused by the conversion of low-LET damage into high-LET damage through an increase in the clustering or severity of lethal DNA lesions caused by irradiation (19, 21).

Other hypotheses have been postulated to explain HP-induced radiosensitization. First, sensitization may be caused by an increase in radiation-induced damage (18). A second hypothesis assumes that effects on repair processes are important, particularly the repair of potentially lethal damage (13). Because both mechanisms focus on

different processes in the cell, both may contribute to radiosensitization (12, 39). In exponentially growing cells, the contribution of the increase in radiation-induced damage by HPs has been reported to be larger than in plateau phase cells (14, 38–40). Because tumors contain both cell populations, investigations of the effectiveness of radiosensitization by HPs in experimental tumor models are needed.

The data presented in this report provide new information on the effect of a clinically relevant hyperthermic treatment of 60 min at 42°C in combination with the incorporation of HPs. We observed in all cell lines that the thermal sensitivity of unirradiated cells increased after exposure to HPs. Others did not observe a significant effect on thermal sensitivity at 42.0 or at 45.5°C after BrdUrd or IdUrd incorporation in V79 cells (29), probably correlated with lower levels of thymidine replacement. However, for synchronized Chinese hamster ovary cells, it has been reported that incorporation of BrdUrd had an additive effect with hyperthermic treatment at 45.5°C (4).

Our data show that HT can further decrease the survival of radiosensitive as well as of radioresistant HP-sensitized cells. It has been shown for otherwise untreated cells that HT had slightly more effect on the β -parameter than on the α -parameter (10). However, our findings indicate that HT primarily induced an increase in the α -parameter, while the effect on the β -parameter was less prominent. It should be noted that the very large values of the linear parameter after treatment of the radiosensitive cell lines often prevented a reliable calculation of the quadratic parameter. The magnitude of the HT-induced increase in the α -parameter was similar for the three HPs studied. Comparison of the increase in the α -parameter of radiosensitive and radioresistant cells indicates that HT might sensitize

Fig. 4. Changes in the linear parameter α of the LQ-formula as a function of the percentage thymidine replacement for radiosensitive R-1 (A) and radioresistant RUC-II (B) cells induced after exposure to 0, 1, 2, and 4 μM CldUrd, BrdUrd, or IdUrd. Hy-

Fig. 5. Overview of the changes in the linear parameter α of the LQ-formula induced by exposure to 4 μM IdUrd with or without hyperthermia (60 min at 42°C). The percentage of thymidine replacement for R-1, RUC-II, MOS, V79, and SW1573 cells were $9.7\% \pm 0.5$, $5.9\% \pm 0.4$, $12.7\% \pm 0.2$, $12.1\% \pm 1.0$, and $10.6\% \pm 0.8$, respectively. Mean values with standard errors of the mean of at least three separate experiments are plotted.

radioresistant cells more. The observation that treatment with HPs and HT of radioresistant RUC-II cells leads to multiplication factors of the α -parameter in excess of 50 suggests that the combined treatment is very promising for highly radioresistant tumors.

As suggested earlier (21, 22), changes in the low dose region relevant to clinical radiotherapy can be analyzed in terms of the sensitizer enhancement ratio at 2 Gy (SER 2 Gy). The changes in the SER 2 Gy induced by various treatments were found to be similar to the changes in the relative increase in the α -parameter (data not shown), indicating that the SER 2 Gy is dominated by the α -parameter of the LQ formalism. When the data on the relative increase in the α -parameter were corrected for the radiosensitizing effect of HT alone, no significant changes in the extent of HP-induced radiosensitization was observed. It has been shown in Chinese hamster V79 cells that BrdUrd and IdUrd incorporation did not alter the sensitivity to a heat dose of 45°C for 5 min (29). Our data support the suggestion that the mechanisms of radiosensitization by HT and incorporation of HPs are not the same (29).

perthermia (60 min at 42°C) was combined with exposure to 4 μM CldUrd, BrdUrd, or IdUrd, and its effect is indicated by arrows. Mean values with standard errors of the mean for at least three separate experiments are plotted. Lines represent linear fits to the data for CldUrd (solid line), BrdUrd (dotted line), and IdUrd (dashed line).

Nonetheless, it is evident that both treatments do not antagonize and, therefore, the combination might be useful in clinical application.

In summary, our findings indicate that HT in combination with the incorporation of HPs additively increases the radiosensitivity of the exponentially growing rodent as well as of human tumor cell lines, reflected by an increase in the linear parameter of the LQ model. In particular, in a cell line with a very low α -value, a large radio-

sensitization factor was obtained. The increasing values of the ratio α/β with increasing sensitization imply that, especially in HP-sensitized cells, the contribution of the quadratic parameter is relatively small, and fractionation effects are expected to be small in the dose range of 1 to 3 Gy. If a rapid repopulation of surviving cells is induced during a course of fractionated radiotherapy, the application of HPs might increase the effectiveness of the last treatments.

REFERENCES

- Aten, J. A.; Bakker, P. J. M.; Stap, J.; Boschman, G. A.; Veenhof, C. H. N. DNA double labelling with IdUrd and CldUrd for spatial and temporal analysis of cell proliferation and DNA replication. *Histochem. J.* 24:251–259; 1992.
- Barendsen, G. W. Dose fractionation, dose rate and iso-effect relationships for normal tissue responses. *Int. J. Radiat. Oncol. Biol. Phys.* 8:1981–1997; 1982.
- Barendsen, G. W.; Broerse, J. J. Differences in radiosensitivity of cells from various types of experimental tumors in relation to the RBE of 15 MeV neutrons. *Int. J. Radiat. Oncol. Biol. Phys.* 3:211–214; 1977.
- Dewey, W. C.; Westra, A.; Miller, H. H.; Nagasawa, H. Heat-induced lethality and chromosomal damage in synchronized chinese hamster cells treated with 5-bromodeoxyuridine. *Int. J. Radiat. Biol.* 20:505–520; 1971.
- Elkind, M. M.; Whitmore, G. F. The influence of chemicals and physical factors on survival. In: Elkind, M. M.; Whitmore, G. F., eds. *The radiobiology of cultured mammalian cells*. New York: Gordon and Breach; 1967:145–235.
- Erikson, E. L.; Szybalski, W. Molecular radiobiology of human cell lines. V. Comparative radiosensitizing properties of 5-halodeoxycytidines and 5-halodeoxyuridines. *Radiat. Res.* 20:252–262; 1963.
- Fowler, J. F.; Kinsella, T. J. The limiting radiosensitization of tumours by S-Phase sensitizers. *Br. J. Cancer* 74(Suppl. 27):S294–S296; 1996.
- Goffman, T. E.; Dachowski, L. J.; Bobo, H.; Oldfield, E. H.; Steinberg, S. M.; Cook, J.; Mitchell, J. B.; Katz, D.; Smith, R.; Glatstein, E. Long-term follow-up on National Cancer Institute Phase I/II study of glioblastoma multiforme treated with iododeoxyuridine and hyperfractionated irradiation. *J. Clin. Oncol.* 10:264–268; 1992.
- Gonzalez Gonzalez, D.; van Dijk, J. D. P.; Blank, L. E. C. M. Radiotherapy and hyperthermia. *Eur. J. Cancer* 31:1351–1355; 1995.
- Haveman, J.; Luinenburg, M.; Wondergem, J.; Hart, A. A. M. Effects of hyperthermia on the linear and quadratic parameters of the radiation survival curve of mammalian cells: Influence of thermotolerance. *Int. J. Radiat. Biol.* 51:561–565; 1987.
- Haveman, J.; Rietbroek, R. C.; Geerdink, A.; Van Rijn, J.; Bakker, P. J. M. Effect of hyperthermia on the cytotoxicity of 2',2'-difluorodeoxycytidine (gemcitabine) in cultured SW1573 cells. *Int. J. Cancer* 62:627–630; 1995.
- Iliakis, G.; Kurtzman, S.; Pantelias, G.; Okayasu, R. Mechanism of radiosensitization by halogenated pyrimidines: Effect of BrdU on radiation induction of DNA and chromosome damage and its correlation with cell killing. *Radiat. Res.* 119:286–304; 1989.
- Iliakis, G.; Wright, E.; Ngo, F. Q. H. Possible importance of PLD repair in the modulation of BrdUrd and IdUrd-mediated radiosensitization in plateau-phase C3H10T1/2 mouse embryo cells. *Int. J. Radiat. Oncol. Biol. Phys.* 51:541–548; 1987.
- Jones, G. D. D.; Ward, J. F.; Limoli, C. L.; Moyer, D. J.; Aguilera, J. A. Mechanisms of radiosensitization in iododeoxyuridine-substituted cells. *Int. J. Radiat. Biol.* 67:647–653; 1995.
- Kooi, M. W.; Stap, J.; Barendsen, G. W. Proliferation kinetics of cultured cells after irradiation with X-rays and 14 MeV neutrons studied by time-lapse cinematography. *Int. J. Radiat. Biol.* 45:583–592; 1984.
- Lawrence, T. S.; Davis, M. A.; Maybaum, J.; Stetson, P. L.; Ensminger, W. D. The dependence of halogenated pyrimidine incorporation and radiosensitization on the duration of drug exposure. *Int. J. Radiat. Oncol. Biol. Phys.* 29:105–113; 1990.
- Lawrence, T. S.; Davis, M. A.; Maybaum, J.; Stetson, P. L.; Ensminger, W. D. The effect of single vs. double-strand substitution on halogenated pyrimidine-induced radiosensitization and DNA strand breakage in human tumor cells. *Radiat. Res.* 123:192–198; 1990.
- Lett, T.; Parkins, G.; Alexander, P.; Ormerod, M. G. Mechanisms of sensitization to X-rays of mammalian cells by 5-bromodeoxyuridine. *Nature* 203:593–596; 1964.
- Lindstadt, D.; Blakely, E.; Phillips, T. L.; Castro, J. R. Radiosensitization produced by iododeoxyuridine with high linear energy transfer heavy ion beams. *Int. J. Radiat. Oncol. Biol. Phys.* 15:703–710; 1988.
- McLaughlin, P. W.; Mancini, W. R.; Stetson, P. L.; Greenberg, H. S.; Nguyen, N.; Seabury, H.; Heidorn, D. B.; Lawrence, T. S. Halogenated pyrimidine sensitization of low dose rate irradiation in human malignant glioma. *Int. J. Radiat. Oncol. Biol. Phys.* 26:637–642; 1993.
- Miller, E. M.; Fowler, J. F.; Kinsella, T. J. Linear-quadratic analysis of radiosensitization by halogenated pyrimidines. I. Radiosensitization of human colon cancer cells by iododeoxyuridine. *Radiat. Res.* 131:81–89; 1992.
- Miller, E. M.; Fowler, J. F.; Kinsella, T. J. Linear-quadratic analysis of radiosensitization by halogenated pyrimidines. II. Radiosensitization of human colon cancer cells by bromodeoxyuridine. *Radiat. Res.* 131:90–97; 1992.
- Nevaldine, J.; Congo, J. A.; Hahn, P. J. Hyperthermia inhibits the repair of DNA double-strand breaks induced by ionizing radiation as determined by pulsed-field gel electrophoresis. *Int. J. Hyperthermia* 10:381–388; 1994.
- O'Dwyer, P. J.; King, S. A.; Hoth, D. F.; Leyland-Jones, B. Role of thymidine in biochemical modulation: A review. *Cancer Res.* 47:3911–3919; 1987.
- Overgaard, J.; Gonzalez Gonzalez, D.; Hulshof, M. C. C. M.; Arcangeli, G.; Dahl, O.; Mella, O.; Bentzen, S. M. Randomised trial of hyperthermia as adjuvant to radiotherapy for recurrent or metastatic malignant melanoma. *Lancet* 345:540–543; 1995.
- Painter, R. B. The role of DNA damage and repair in killing induced by ionizing radiation. In: Meyn, R. E.; Withers, H. R., eds. *Radiation biology in cancer research*. New York: Raven Press; 1980:59–68.

27. Phillips, T. L.; Bodell, W. J.; Uhl, V.; Ross, G. Y.; Rasmussen, J.; Mitchell, J. B. Correlation between exposure time, concentration and incorporation of IdUrd in V-79 cells with radiation response. *Int. J. Radiat. Oncol. Biol. Phys.* 16:1251–1255; 1989.
28. Phillips, T. L.; Scott, C. B.; Leibel, S. A.; Rotman, M.; Weigensberg, I. J. Results of a randomized comparison of radiotherapy and bromodeoxyuridine with radiotherapy alone for brain metastases: Report of RTOG trial 89-05. *Int. J. Radiat. Oncol. Biol. Phys.* 33:339–348; 1995.
29. Raaphorst, G. P.; Vadasz, J. A.; Azzam, E. I. Thermal sensitivity and radiosensitization in V79 cells after BrdUrd or IdUrd incorporation. *Radiat. Res.* 98:167–175; 1984.
30. Rodriguez, R.; Ritter, M. A.; Fowler, J. F.; Kinsella, T. J. Kinetics of cell labeling and thymidine replacement after continuous infusion of halogenated pyrimidines *in vivo*. *Int. J. Radiat. Oncol. Biol. Phys.* 29:105–113; 1994.
31. Sullivan, F. J.; Herscher, L. L.; Cook, J. A.; Smith, J.; Steinberg, S. M.; Epstein, A. H.; Oldfield, E. H.; Goffman, T. E.; Kinsella, T. J.; Mitchell, J. B.; Glatstein, E. National Cancer Institute (Phase II) study of high-grade glioma treated with accelerated hyperfractionated radiation and iododeoxyuridine: Results in anaplastic astrocytoma. *Int. J. Radiat. Oncol. Biol. Phys.* 30:583–590; 1994.
32. Szybalski, W. X ray sensitization by halopyrimidines. *Cancer Chemother. Rep.* 58:539–557; 1974.
33. Thames, H. D.; Withers, H. R.; Peters, L. J.; Fletcher, G. H. Changes in early and late radiation responses with altered dose fractionation: Implications for dose–survival relationships. *Int. J. Radiat. Oncol. Biol. Phys.* 8:219–226; 1982.
34. Uhl, V.; Phillips, T. L.; Ross, G. Y.; Bodell, W. J.; Rasmussen, J. Iododeoxyuridine incorporation and radiosensitization in three human tumor cell lines. *Int. J. Radiat. Oncol. Biol. Phys.* 22:489–494; 1992.
35. Urtasun, R. C.; Kinsella, T. J.; Farnan, N.; Delrowe, J. D.; Lester, S. G.; Fulton, D. S. Survival improvement in anaplastic astrocytoma, combining external radiation with halogenated pyrimidines: Final report of RTOG 86-12, phase I–II. *Int. J. Radiat. Oncol. Biol. Phys.* 36:1163–1167; 1996.
36. Van Bree, C.; Rietbroek, R. C.; Schopman, E. M.; Kipp, J. B. A.; Bakker, P. J. M. 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.
37. Van Bree, C.; Krooshoop, J. J.; Rietbroek, R. C.; Kipp, J. B. A.; Bakker, P. J. M. Hyperthermia enhances tumor uptake and antitumor efficacy of thermostable liposomal daunorubicin in a rat solid tumor. *Cancer Res.* 56:563–568; 1996.
38. Wang, Y.; Iliakis, G. Effect of 5'-iododeoxyuridine on the repair of radiation induced potentially lethal damage interphase chromatin breaks and DNA double strand breaks in Chinese hamster ovary cells. *Int. J. Radiat. Oncol. Biol. Phys.* 23:353–360; 1992.
39. Wang, Y.; Pantelias, G. E.; Iliakis, G. Mechanisms of radiosensitization by halogenated pyrimidines: The contribution of excess in DNA and chromosome damage may be minimal in plateau cells. *Int. J. Radiat. Biol.* 66:133–142; 1994.
40. Webb, C. F.; Jones, G. D. D.; Ward, J. F.; Moyer, D. J.; Aguilera, J. A.; Ling, L. L. Mechanisms of radiosensitization in bromodeoxyuridine substituted cells. *Int. J. Radiat. Biol.* 64:695–705; 1993.