Radiosensitization by bromodeoxyuridine and hyperthermia: analysis of linear and quadratic parameters of radiation survival curves of two human tumor cell lines

Franken, N.A.P.; van Bree, C.; Veltmaat, M.A.T.; Rodermond, H.M.; Haveman, J.; Barendsen, G.W.

DOI
10.1269/jrr.42.179

Publication date
2001

Published in
Journal of Radiation Research

J. RADIAT. RES., 42, 179–190 (2001)

Radiosensitization by Bromodeoxyuridine and Hyperthermia: Analysis of Linear and Quadratic Parameters of Radiation Survival Curves of Two Human Tumor Cell Lines

NICOLAAS A. P. FRANKEN[1]*, CHRIS VAN BREE[1], MARTIJN A. T. VELTMAAT[1], HANS M. RODERMOND[1], JAAP HAVEMAN[1] and GERRIT W. BARENDSEN[1].

[1]Department of Radiotherapy, Academic Medical Center, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands.

(*Received on October 11, 2000*)
(*Revision received on February 16, 2001*)
(*Accepted on April 11, 2001*)

LQ analysis/Bromodeoxyuridine (BrdUrd)/Hyperthermia/Radiosensitization/Human tumor cells.

Sensitization by bromodeoxyuridine (BrdUrd) and hyperthermia (HT) on cell reproductive death induced by ionizing radiation was analyzed using the linear-quadratic [$S(D)/S(0)=exp\{-(\alpha D+\beta D^2)\}$] model. Plateau-phase human lung tumor cells (SW-1573) and human colorectal carcinonoma cells (RKO) were treated with BrdUrd, radiation and HT. LQ-analysis was performed at iso-incubation dose and at iso-incorporation level of BrdUrd, and at iso-HT doses and iso-survival levels after HT. Clonogenic assays were performed 24 h after treatment to allow repair of potentially lethal damage (PLD). In SW cells BrdUrd, HT or the combination significantly increased the α-parameter (factor 2.0-5.7), without altering the β-parameter. In RKO cells sensitization with BrdUrd increased both α (factor 1.4) and β (factor 1.3) while HT only influenced β (factor 2.1–4.0). The combination did not further increase the α and β. The results indicate that BrdUrd has its main effect on the parameter α, dominant at clinically relevant radiation doses but that HT can affect both α and β. The addition of BrdUrd and HT provides a method to enhance the efficacy of radiotherapy.

INTRODUCTION

The incorporation of halogenated pyrimidines (HP's) into the DNA is known to increase the radiosensitivity of mammalian cells *in vitro* and *in vivo*. The HP's bromo-deoxyuridine (BrdUrd) and iodo-deoxyuridine (IdUrd) are already applied clinically to enhance loco-regional effectiveness of radiotherapy[1–3]. The level of radiosensitization by HP's has been shown to correlate with the degree of thymidine-replacement[4–6]. Cells that have incorporated

*Corresponding author: Phone; ++ 20 566 8769, Fax: ++ 20 609 1278, Email: N.A.Franken@amc.uva.nl

Abbreviations: LQ: linear quadratic; BrdUrd: bromodeoxyuridine; HT: hyperthermia; PLD: potentially lethal damage

HP's demonstrate an increase in the amount of radiation-induced DNA double-strand breaks[7,8] and chromosomal aberrations[9,10].

Hyperthermia (HT) is also known to sensitize cells to radiation and clinical studies have demonstrated that it is beneficial in combination with radiotherapy[11–13]. It has been reported that HT inhibits repair of DNA double-strand breaks[14,15] that are suggested to be the lesions by which radiation kills cells[16].

The combination of HP's and HT might increase the effectiveness of radiotherapy. Cells that have not incorporated HP's might be located in poorly vascularized tumor areas where cells are more sensitive to HT. Moreover the inhibitory effect of HT on DNA DSB repair could be extra beneficial on BrdUrd sensitized cells. However, previous studies have not demonstrated a synergististic effect of HP-incorporation and HT[4,17].

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 (LQ) model[18–21]. The LQ-model leads to a description of survival curves by the formula: $S(D)/S(0) = \exp\{-(\alpha D + \beta D^2)\}$[22–25]. The parameters, α and β, are assumed to reflect specific mechanisms of cell killing by radiation. The linear term dominates the response at low doses and the quadratic term plays a major role at high doses. An increase of α has been suggested to be due to enhanced expression of potentially lethal damage (PLD). An increase of β suggests an enhanced contribution due to interaction of sublethal damage (SLD)[24]. Independent of suggestions about biological mechanisms, using the LQ-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 of HP's, especially in the dose range of 1 to 3 Gy as commonly applied in fractionated radiotherapy.

Several publications have appeared on the use of the LQ model on radiation modifying agents and results have been somewhat contradictory[26–28]. Hartson-Eaton *et al.*[26] observed an effect of HT mainly on the value of α of exponentially growing CHO cells. Holahan *et al.*[27] observed an increase of both α and β in exponentially growing and G_1-phase CHO cells. In these studies hyperthermia was given before irradiation. Haveman *et al.*[28] studying survival curves of exponentially growing M8013 murine cells, observed that HT predominantly increased the value of β. Irradiation was applied halfway during the HT treatment. Van Bree *et al.*[4] studied the effect of HT (applied after irradiation) and HP's on survival curves of several exponentially growing rodent and human tumor cell lines with different radiosensitivity, and observed an effect mainly on the value of the α. In studies on the radiosensitization of exponentially growing human colon cancer cell lines by incorporation of the HP's only, it has been shown that the linear term is strongly increased, but that the quadratic term is hardly affected[18–21]. In most cited studies (except Holahan *et al.*[27]) on HT and/or HP radiosensitization, experiments were performed on exponentially growing cells and cells were plated for clonogenic assay immediately after the last treatment. However, most tumors contain quiescent clonogenic cells and their response must be studied as well. Roy *et al.*[29] observed repair of PLD after delayed plating as compared with immediate plating of irradiated non-sensitized G0 human embryo cells (HE60). In delayed plated HP-radiosensitized plateau

phase V79 cells PLD repair was decreased as evidenced by an increase of α, which in all treatment groups was observed[5]. The β increased only if cells were plated immediately after treatment.

In the present study the effects of BrdUrd, at iso-incubation dose and iso-incorporation level, and/or HT, at iso dose and iso survival level, are examined on the LQ parameters of the radiation survival curves with delayed plated plateau-phase human lung carcinoma cells (SW-1573) and human colon carcinoma cells (RKO). Plateau-phase cells were studied as these cultures have certain characteristics similar to quiescent cells in tumors[30]. We deduced that BrdUrd and/or HT treatment differently affected the LQ-parameters of both cell lines.

MATERIAL AND METHODS

Cell culture

The human squamous lung carcinoma cell line SW-1573 is grown at 37°C as monolayers in 75 cm^2 tissue culture flasks (Costar/Corning) in Leibovitz-15 medium (L-15, Gibco-BRL) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. The L-15 medium does not require CO_2. The doubling time of the cells during exponential growth is 23 h[6].

The human colon cancer cell line RKO is grown at 37°C as monolayers in 25 or 75 cm^2 tissue culture flasks (Costar) in McCoy's 5A medium + 25 mM Hepes (Gibco/BRL) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. McCoy's 5A medium requires 5% CO_2. The doubling time of the cells during exponential growth is 24 h[31].

Treatment

For experiments, cells grew for 48 h in the absence or presence of bromodeoxyuridine (BrdUrd) (Sigma). The SW-1573 cells were incubated with 0, 1 and 4 μM and the RKO cells with 0 and 4 μM of BrdUrd. The experiments with SW-1573 cells incubated with 1 μM BrdUrd were carried out in order to obtain similar incorporation levels of BrdUrd in the DNA as in the RKO cells grown in 4 μM BrdUrd. Cells were grown until plateau-phase and subsequently irradiated with different doses up to 8 Gy with a ^{137}Cs-source at a dose-rate of about 0.8 Gy/min.

Hyperthermia (HT) was applied in a thermostatically controlled waterbath positioned in an incubator. SW cells were treated for 60 min at 41.0°C, and RKO cells were treated for 15 min or 60 min at 41.0°C. The 15 min heat treatment of RKO cells resulted in similar survival levels as of SW cells treated for 60 min at 41.0°C. During HT treatment of the RKO cells, CO_2 was applied. In case of combined treatment, i.e. irradiation and HT, cells were irradiated first and directly thereafter placed in the waterbath for HT treatment.

Clonogenic assay

Twenty-four hours after treatment cells were harvested and replated in appropriate dilu-

tions in 6-wells macroplates (Greiner). Ten days after inoculation, the colonies were fixed and stained in 6% glutaraldehyde with 0.05% crystalviolet. Colonies of 50 cells or more were scored as originating from a single clonogenic cell. The plating efficiencies of untreated SW-1573 and RKO cells are about 90% and 40% respectively. Results of three separate experiments were used for analysis of cell survival. Survival curves were fitted to the data according to the formula $S(D)/S(0) = exp\{-(\alpha D + \beta D^2)\}$[22].

BrdUrd-incorporation

Percentage of thymidine-replacement was measured by the technique described by Belanger *et al.*[32] and Franken *et al.*[5,6]. With flow cytometry the labeling index of the cells was checked.

RESULTS

Effects on cell growth and percentage of thymidine replacement by BrdUrd.

The incorporation of BrdUrd did neither result in any growth delay nor was the plating efficiency affected. After incubation with 1 or 4 μM BrdUrd the percentage of thymidine-replacement in the DNA of SW-1573 at the time of irradiation was 6.7 ± 0.5% and 19.5 ± 0.5% respectively. In RKO cells the percentage of thymidine replacement after incubation with 4 μM of BrdUrd was 7.1 ± 0.8%. Flowcytometric analysis demonstrated that at time of irradiation all cells were labeled either after incubation with 1 μM and after incubation with 4 μM BrdUrd.

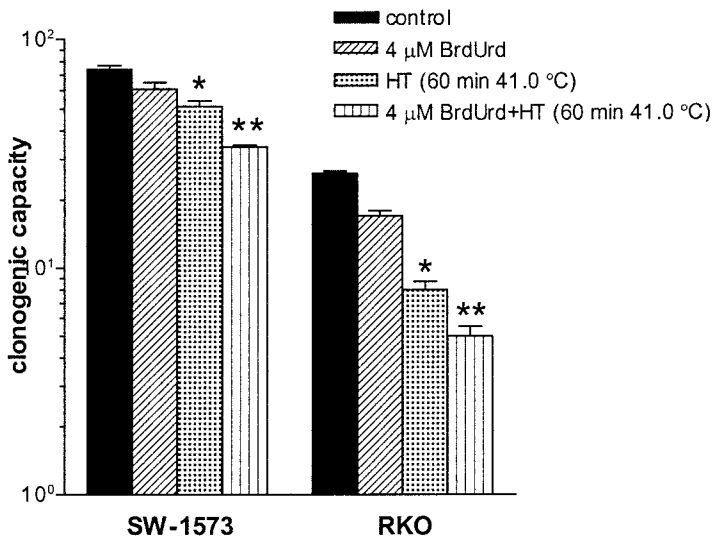

Fig. 1. Survival of SW-1573 and RKO cells after treatment with 4 mM BrdUrd, hyperthermia 60 min at 41.0°C and combined BrdUrd/Hyperthermia. Mean results of three separate experiments ± SEM. (* significantly different from control $P < 0.05$, ** significantly different from HT only $P < 0.05$)

Clonogenic survival of SW-1573 cells and RKO cells after BrdUrd and HT treatment without irradiation

The clonogenic capacity of SW-1573 cells after treatment with 4 μM BrdUrd alone, HT (41.0°C for 60 min) alone, and combined 4 μM BrdUrd/HT (41.0°C for 60 min) decreased to respectively 84%, 70% and 55% of controls (Fig. 1). The clonogenic capacity of the SW-1573 cells after treatment with 1 μM BrdUrd alone and combined 1 μM BrdUrd/HT respectively, did not differ significantly from controls and HT alone respectively (data not shown). The clonogenic capacity of RKO cells after treatment with BrdUrd only, HT (41.0°C for 60 min) only and combined BrdUrd/HT (41.0°C for 60 min) decreased to respectively 65%, 31% and 19% of controls. The clonogenic capacity of RKO cells HT treated for 15 min at 41.0°C and combined 4 μM BrdUrd/HT (41.0°C for 15 min), decreased to about 70% and 52% respectively. As is shown in Fig. 1, HT (41.0°C for 60 min) alone resulted in a significantly reduced cell survival compared to controls and the combined 4 μM BrdUrd/HT (41.0°C for 60 min) treatment resulted in a significantly reduced cell survival as compared to HT (41.0°C for 60 min) alone treatment. Surviving fractions after irradiation were corrected for the decrease in plating efficiency.

Clonogenic survival of SW-1573 cells after irradiation and treatment with BrdUrd and HT

Survival curves of the human SW-1573 cells are shown in Fig. 2. (For clarity the sur-

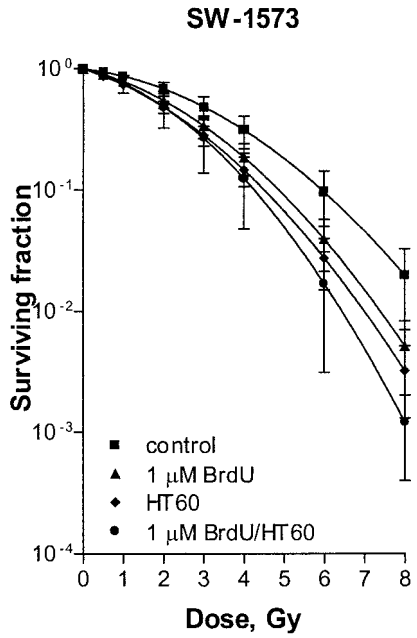

Fig. 2. Radiation dose-survival curves of SW-1573 cells: no radiosensitization (■); after radiosensitization with 1 μM BrdUrd (▲); after radiosensitization with hyperthermia (60 min at 41.0°C) (◆); after sensitization with 1 μM BrdUrd and hyperthermia (60 min at 41.0°C) (●). Mean results of three separate experiments ± SEM.

vival curves after treatment with 4 μM BrdUrd alone and combination 4 μM BrdUrd/HT are omitted from the graph). The values of α, the parameter of the linear term determining the initial slope, and the value of β, the parameter of the quadratic term determining the continuously curving high dose region, of the survival curves of this cell line are presented in Table 1. BrdUrd and/or HT induced α- and β-enhancement factors are presented in Fig. 3. The parameter a increased by a factor 2.0 and 3.2 when cells were radiosensitized with 1 and 4 μM BrdUrd. The a increased by a factor respectively 2.7 when radiation was followed by HT, and by a factor 2.2 and 5.7 respectively, when radiosensitization with 1 or 4 μM BrdUrd was

Table 1. LQ parameters α and β for clonogenic survival after sensitization of SW-1573 cells with BrdUrd, HT or BrdUrd+HT.

Treatment	α, Gy^{-1}	β, Gy^{-2}
control	0.09 ± 0.05	0.05 ± 0.01
1 μM BrdUrd	0.18 ± 0.03	0.06 ± 0.01
4 μM BrdUrd	0.29 ± 0.08	0.06 ± 0.02
HT (60 min 41.0°C)	0.24 ± 0.04	0.06 ± 0.01
1 μM BrdUrd/HT (60 min 41.0°C)	0.20 ± 0.05	0.07 ± 0.01
4 μM BrdUrd/HT (60 min 41.0°C)	0.51 ± 0.07	0.04 ± 0.02

The values are mean results of three independent experiments ± S.D.

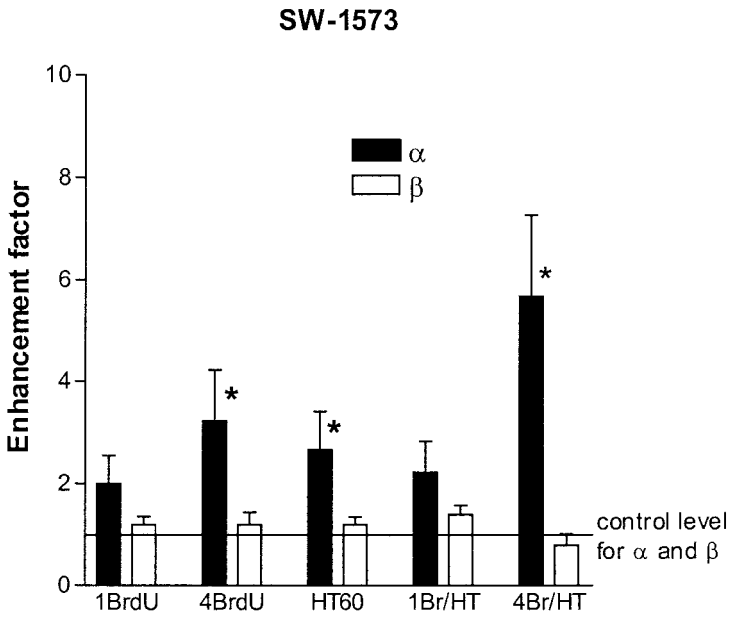

Fig. 3. α and β enhancement factors of SW 1573 cells. 1BrdU: 1 μM BrdUrd; 4BrdU: 4 μM BrdUrd; HT60: HT 60 min at 41.0°C; 1Br/HT: 1 μM BrdUrd combined with HT 60 min at 41.0°C; 4Br/HT: 4 μM BrdUrd combined with HT 60 min at 41.0°C. Mean results of three separate experiments ± SEM. (* significantly different from control level $P < 0.05$).

followed by HT. The parameter β was not significantly affected by the sensitizing treatments.

Clonogenic survival of RKO cells after irradiation and treatment with BrdUrd and HT

Survival curves of the human RKO cell line are shown in Fig. 4, (survival curves after treatment with HT for 60 min at 41.0°C and combination 4 μM BrdUrd/HT 60 min at 41.0°C are omitted for clarity). The values obtained for the parameters α and β after analysis of the survival curves using the LQ model are shown in Table 2. The enhancement factors are pre-

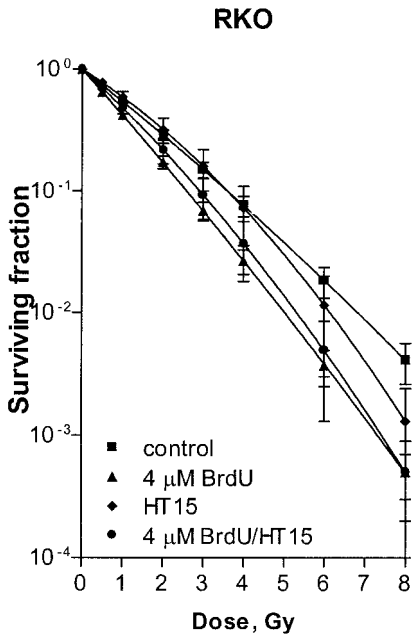

Fig. 4. Radiation dose-survival curves of RKO cells: no radiosensitization (■); after radiosensitization with 4 μM BrdUrd (▲); after radiosensitization with hyperthermia (15 min at 41.0°C) (◆); after sensitization with 4 μM BrdUrd and hyperthermia (15 min at 41.0°C) (●). Mean results of three separate experiments ± SEM.

Table 2. LQ parameters α and β for clonogenic survival after sensitization of RKO cells with BrdUrd, HT or BrdUrd+HT.

Treatment	α, Gy^{-1}	β, Gy^{-2}
Control	0.60 ± 0.04	0.011 ± 0.007
4 μM BrdUrd	0.85 ± 0.09	0.014 ± 0.009
HT (15 min 41.0°C)	0.48 ± 0.09	0.044 ± 0.022
HT (60 min 41.0°C)	0.58 ± 0.09	0.023 ± 0.010
4 μM BrdUrd/HT (15 min 41.0°C)	0.70 ± 0.09	0.031 ± 0.022
4 μM BrdUrd/HT (60 min 41.0°C)	0.96 ± 0.09	0.040 ± 0.019

The values are mean results of three independent experiments ± S.D.

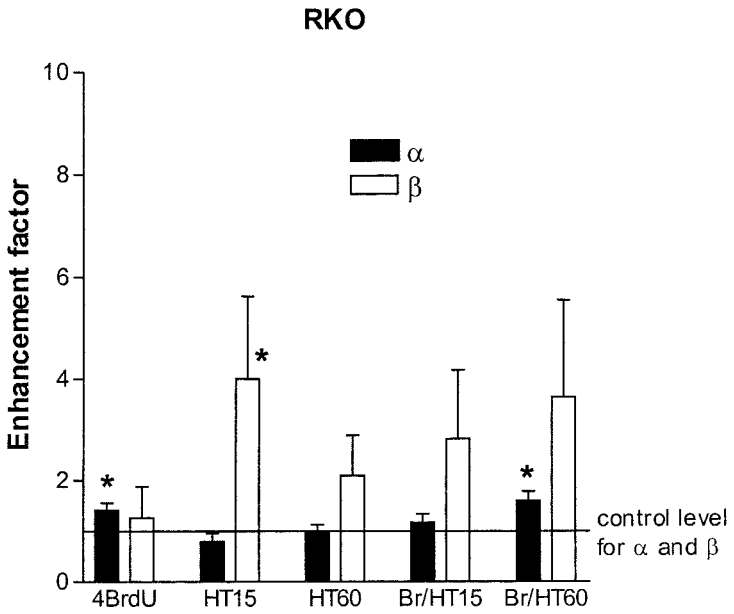

Fig. 5. α and β enhancement factors of RKO cells. 4BrdU: 4 μM BrdUrd; HT15: HT 15 min at 41.0°C; HT60: HT 60 min at 41.0°C; Br/HT15: 4 μM BrdUrd combined with HT 60 min at 41.0°C; Br/HT60: 4 μM BrdUrd combined with HT 60 min at 41.0°C. Mean results of three separate experiments ± SEM. (* significantly different from control level $P < 0.05$)

sented in Fig. 5. The increase of α after sensitization with BrdUrd only, BrdUrd/HT 15 min 41.0°C and BrdUrd/HT 60 min 41.0°C is a factor 1.4, 1.2 and 1.6, respectively. HT only did not increase the α parameter. No significant difference was observed after HT at 41.0°C for 15 min or for 60 min in the LQ parameters of RKO cells with or without BrdUrd incorporation. The quadratic parameter, β, is influenced by sensitization with BrdUrd and/or HT in RKO cells. The increase of β after BrdUrd only, HT60, BrdUrd/HT 15 min 41.0°C and BrdUrd/HT 60 min 41.0°C was not significant. The value of β after treatment of cells with 4 μM BrdUrd increased by a factor of 1.3. After HT treatment only the β increased by a factor 2.1–4.0. The combination of BrdUrd and HT did not further increase this value.

DISCUSSION

After incubation with equal concentrations of BrdUrd (4 μM) the SW-1573 cells incorporated higher levels of BrdUrd into the DNA than the RKO cells. High levels of HP incorporation might be due to a deficiency in the mismatch repair pathway[33]. But the status of this repair pathway in the studied cell lines is not known. However, additional experiments with the SW-1573 cells were carried out with 1 μM BrdUrd. This concentration resulted in approximately similar incorporation levels as the RKO cells after 4 μM BrdUrd. The increase

of α after BrdUrd-induced radiosensitization, even at iso-incorporation levels, in the relatively radioresistant SW-1573 cells is more pronounced than in the radiosensitive RKO cells (Table 1 and 2). In the RKO cells also an effect on β is observed although it should be noted that the value of β has large uncertainties. Results of earlier studies by Van Bree *et al*.[4], Franken *et al*.[5,6] and Miller *et al*.[20,21] indicated that HP incorporation increases the value of α and that radioresistant cell lines are more sensitized by HP's than radiosensitive cell lines.

It is also shown that in different types of cell lines derived from human tumours mild HT (15 min-60 min at 41.0°C) enhances the radiation effects differently. At iso-survival level after HT in the SW-1573 cells (60 min at 41.0°C) the α increased while in the RKO cells after HT (15 min at 41.0°C) the β increased. Different mechanisms of action have been described to account for HT induced radiosensitization[15]. The increase of α suggests inhibition of repair of PLD and the increase of β suggests inhibition of repair of SLD.

Incorporation of BrdUrd does not increase the thermal sensitivity of unirradiated cells. The combination of HT (41.0°C for 60 min) with 1 or 4 μM BrdUrd resulted in lower surviving fractions than of both treatments alone, but the effect was merely additive. An additive effect of BrdUrd and HT (45.5°C) was also reported by Dewey *et al*.[34] for synchronized hamster cells. HP-induced thermal sensitization was found by Van Bree *et al*.[4] in different exponentially growing cell lines after hyperthermia treatment at 42.0°C for 1 hour. In contrast with these data are results of Raaphorst *et al*.[17], who did not observe a significant effect on thermal sensitivity at 42.0 or at 45.5°C after BrdUrd or IdUrd incorporation in synchronized V79 cells.

Our data show that HT can further increase radiation damage of BrdUrd-sensitized cells and this is observed even in quiescent clonogenic cells. Of the somewhat radioresistant SW-1573 the value of α and of the more radiosensitive RKO cells the value of β increased after combined sensitization. In the study by Van Bree *et al*.[4] in several different exponentially growing cell lines including the SW cells, after hyperthermic treatment at 42.0°C for 1 hour only an effect on the value of α was observed. However, in otherwise untreated M8010 cells Haveman *et al*.[28] showed a clear effect on the value of β. It can be argued that HT modifies the radiation response of the various cell lines via different mechanisms or pathways. HT inhibits all kinds of DNA repair processes like repair of radiation-induced single and double strand breaks and base excision repair and also DNA polymerase activity.

The description of combined effects of radiation and other agents such as hyperthermia may require considerations of complex interactions. A theoretical model for describing the effect of multiple types of radiation applied simultaneously has been developed by Susuki[35]. In this model q(t) is included as an extra parameter for the time of irradiation. For very long treatment times this reduction factor is 0. In our studies treatments were applied sequentially. Moreover, in our experiments survival was studied at 24 hours after the last treatment in order to allow repair of PLD. As HT affects repair processes, this repair time should be included in the treatment time and this ads to the complexity of responses. Therefore the value of q(t) is unknown. The LQ-model in which survival curves are described by the two parameters α and β[23], as used in our study, seems appropriate as a first approximation. HT can influence both parameters. Inhibition of repair of potentially lethal lesions mainly causes an increase of the

linear parameter, α. Inhibition of repair of sublethal lesions (possibly associated with DNA double strand breaks) causes an increase of the quadratic parameter, β.

With regard to clinical implications, it is of interest that the more radioresistant cell line, even in quiescent phase, is sensitized in the low dose region. It can also be suggested that when HT is combined with low dose or fractionated radiotherapy, a substantial enhancement of the effectiveness of irradiation may be expected in case the α parameter is increased. In case the β parameter is increased, HT may not be very effective in modifying radioresponse after irradiation with low doses per fraction. Incorporation of halogenated pyrimidines in combination with HT further increases radiosensitivity. Therefore this combination offers a useful perspective for clinical application.

ACKNOWLEDGEMENTS

This work was financially supported by the J.A. Cohen Institute, Interuniversity Research Institute for Radiopathology and Radiation Protection (grant # 7.1.7.). The Maurits and Anna de Kock foundation is acknowledged for sponsoring laboratory equipment.

REFERENCES

1. 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. and Glatstein, E. (1994) 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.
2. Urtasun, R. C., Kinsella, T. J., Farnan, N., Delrowe, J. D., Lester, S. G. and Fulton, D. S. (1996) 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.
3. Williams, J. A., Yuan, X., Dillehay, L. E., Shastri, V. R., Brem, H. and Williams, J. R. (1998) Synthetic, implantable polymers for local delivery of IUDR to experimental human malignant glioma. Int. J. Radiat. Oncol. Biol. Phys. **42**: 631–639.
4. Van Bree, C., Franken, N. A. P., Bakker, P. J. M., Klomp-Tukker, L. J., Barendsen, G. W. and Kipp, J. B. A. (1997) Hyperthermia and incorporation of halogenated pyrimidines: Radiosensitization in cultured rodent and human tumor cells. Int. J. Radiat. Oncol. Biol. Phys. **39**: 489–496.
5. Franken, N. A. P., Van Bree, C., Kipp, J. B. A. and Barendsen, G. W. (1997) Modification of potentially lethal damage in irradiated chinese hamster V79 cells after incorporation of halogenated pyrimidines. Int. J. Radiat. Biol. **72**: 101–109.
6. Franken, N. A. P., Van Bree, C., Streefkerk, J. O., Kuper, I. M. J. A., Rodermond, H. M. Kipp, J. B. A., Haveman, J. and Barendsen, G. W. (1997) Radiosensitization by Iodo-deoxyuridine in cultured SW-1573 human lung tumor cells: Effects on α and β of the linear-quadratic model. Oncol. Rep. **4**: 1073–1076.
7. Webb, C. F., Jones, G. D. D., Ward, J. F., Moyer, D. J., Aguilera, J. A. and Ling, L. L. (1993) Mechanisms of radiosensitization in bromodeoxyuridine substituted cells. Int. J. Radiat. Biol. **64**: 695–705.
8. Wang, Y., Pantelias, G. E. and Iliakis, G. (1994) 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.

9. Franken, N. A. P., Ruurs, P., Ludwików, G., Van Bree, C., Kipp, J. B. A., Darroudi, F. and Barendsen, G. W. (1999), Correlation between cell reproductive death and chromosome aberrations assessed by FISH for low and high doses of radiation and sensitization by iododeoxyuridine in human SW-1573 cells. Int. J. Radiat. Biol. **75**: 293–299.
10. Franken, N. A. P., van Bree, C., Veltmaat, M. A. T., Ludwików, G., Kipp, J. B. A. and Barendsen, G. W. (1999) Increased chromosome exchange frequencies in iodo-deoxyuridine-sensitized human SW-1573 cells after γ-irradiation. Oncol. Rep. **6**: 59–63.
11. Terashima, H.I., Hiraoka, M., Nishimura, Y., Imajo, Y., Hiratsuka, J., Karasawa, K., Kitahara, T., Kataoka, M., Wada, S. And Hashida, I. (1996) Multi-institutional clinical study on hyperthermia combined with radiotherapy in lung cancer. A retrospective analysis by JASTRO hyperthermia study group. In: Hyperthermic Oncology vol II, Proc. of the 7th International Congress on Hyperthermic Oncology, Roma Italy, Eds. C. F. G. Arcangeli and R. Cavaliere, pp 81–82, Tor Vergata, Italy.
12. Van Der Zee, J., González González, D., Van Rhoon, G. C., Van Dijk, J. P. D., Van Putten, W. J. L., Hart, A.A.M. and the Dutch Deep Hyperthermia Group (2000) Comparison of radiotherapy alone with radiotherapy plus hyperthermia in locally advanced pelvic tumours: a prospective, randomised, multicentre trial. Lancet **355**: 1119–1125.
13. González González, D., van Dijk, J. D. P. and Blank, L. E. C. M. (1995) Radiotherapy and hyperthermia. Eur. J. Cancer **31**: 1351–1355.
14. Nevaldine, J., Congo, J. A. and Hahn, P. J. (1994) 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.
15. Van Bree, C. (1996) Preclinical studies on hyperthermia-enhanced effectiveness of antitumor treatments. Thesis University of Amsterdam.
16. Painter, R. B. (1980) The role of DNA damage and repair in killing induced by ionizing radiation. In: Radiation Biology in Cancer Research, Eds. R. E. Meyn and H. R. Withers, pp. 59–68: Raven Press, New York.
17. Raaphorst, G. P., Vadasz, J. A. and Azzam, E. I. (1984) Thermal sensitivity and radiosensitization in V79 cells after BrdUrd or IdUrd incorporation. Radiat. Res. **98**: 167–175.
18. Lawrence, T. S., Davis, M. A., Maybaum, J., Stetson, P. L. and Ensmiger, M. D. (1990) 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.
19. McLaughlin, P. W., Mancini, W. R., Stetson, P. L., Greenberg, H. S., Nguyen, N., Seabury, H., Heidorn, D. B. and Lawrence, T. S. (1993) Halogenated pyrimidine sensitization of low dose rate irradiation in human malignant glioma. Int. J. Radiat. Oncol. Biol. Phys. **26**: 637–642.
20. Miller, E. M., Fowler, J. F. and Kinsella, T. J. (1992) Linear-quadratic analysis of radiosensitization by halogenated pyrimidines. 1. Radiosensitization of human colon cancer cells by iododeoxyridine. Radiat. Res. **131**: 81–89.
21. Miller, E. M., Fowler, J. F. and Kinsella, T. J. (1992) Linear-quadratic analysis of radiosensitization by halogenated pyrimidines. II. Radiosensitization of human colon cancer cells by bromodeoxyuridine. Radiat. Res. **131**: 90–97.
22. Barendsen, G. W. (1982) Dose fractionation, dose rate and iso-effect relationships for normal tissue responses. Int. J. Radiat. Oncol. Biol. Phys. **3**: 1981–1997.
23. Barendsen, G. W. (1990) Mechanisms of cell reproductive death and shapes of radiation dose-survival curves of mammalian cells. Int. J. Radiat. Biol. **57**: 885–896.
24. Barendsen, G. W. (1997) Parameters of linear-quadratic radiation dose-effect relationships: dependence on LET and mechanisms of reproductive cell death. Int. J. Radiat. Biol. **71**: 649–655.
25. Takatsuji, T., Yoshikawa, I. and Sasaki, M.S. (1999) Generalized concept of the LET-RBE relationship of radiation-induced chromosome aberration and cell death. J. Radiat. Res. **40**: 59–69.
26. Hartson-Eaton, M., Malcolm, A. W. and Hahn, G. M. (1984) Radiosensitivity and thermosensitization of thermotolerant Chinese hamster cells and rif-1 tumors. Radiat. Res. **99**: 175–184.

27. Holahan, E. V., Highfield, D. P., Holahan, P. K. and Dewey, W. C. (1984) Hyperthermic killing and hyperthermic radiosensitization in Chinese hamster ovary cells: Effects of pH and thermal tolerance. Radiat. Res. **97**: 108–131
28. Haveman, J., Luinenburg, M., Wondergem, J., Hart, A. A. M. (1987) Effects of hyperthermia on the linear and quadratic parameters of the radiation survival curve of mammalian cells. Int. J. Radiat. Biol. **51**: 561–565.
29. Roy, K., Kodama, S., Suzuli, K. and Watanabe, M. (1999) Delayed cell death, giant cell formation and chromosome instability induced by X-irradiation in human embryo cells. J Radiat. Res. **40**: 311–322.
30. Hahn, G. M. and Little, J. B. (1972) Plateau-phase cultures of mammalian cells: An in vitro model of human cancer. Curr. Top. Radiat. Res. Quat. **8**: 39–83.
31. Van Bree, C., Van der Maat, B., Ceha, H. M., Franken, N. A. P., Haveman J. and Bakker, P. J. M. (1999) Inactivation of p53 and of pRb protects human colorectal carcinoma cells against hyperthermia-induced cytotoxicity and apoptosis. J. Cancer Res. Clin. Oncol. **125**: 549–555.
32. Belanger, K., Collins, J. M., and Klecker, R. W. (1987) Technique for detection of DNA nucleobases by reversed-phase high performance liquid chromatography optimized for quantitative determination of thymidine substitution by iododeoxyuridine. J. Chromat. **417**: 57–63.
33. Berry, S. E., Garces, C., Hwang, H.-S., Kunugi, K., Meyers, M., Davis, T. W., Boothman, D.A. and Kinsella, T.J. (1999) The mismatch repair protein, hMLH1, mediates 5-substituted halogenated thymidine analogue cytotoxicity, DNA incorporation, and radiosensitization in human colon cancer cells. Cancer Res. **59**: 1840–1845.
34. Dewey, W. C., Westra, A., Miller, H. M. and Nagasawa, H. (1971) Heat-induced lethality and chromosomal damage in synchronized chinese hamster cells treated with 5-bromodeoxyuridine. Int. J. Radiat. Biol. **20**: 505–520.
35. Suzuki, S. (1998) A theorethical model for simultaneous mixed irradiation with multiple types of radiation. J Radiat. Res. **39**: 215–221.