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Radiosensitization with Hyperthermia and Chemotherapeutic Agents: Effects on Linear-Quadratic Parameters of Radiation Cell Survival Curves

Nicolaas A. P. Franken et al.*

*Academic Medical Centre, University of Amsterdam,
Laboratory for Experimental Oncology and Radiobiology (LEXOR),
Centre for Experimental Molecular Medicine, Department of Radiation Oncology,
The Netherlands*

1. Introduction

Radiosensitization effects of hyperthermia and chemotherapeutic agents as currently exploited in the clinic are discussed with respect to the linear quadratic parameters of dose-survival curve presentations. Studies of different human tumour cell lines show that a synergistic interaction can be obtained between hyperthermia, chemotherapy and radiation and that this interaction is more likely to occur in cell lines which are relatively sensitive to chemotherapy. The influence of modifying agents on radiation dose survival curves can adequately be analysed with the use of the linear-quadratic model: $S(D)/S(0) = \exp(-\alpha D + \beta D^2)$. The linear parameter, α , represents lethal damage from single particle events and describes the low dose area while the quadratic parameter, β , indicating sub lethal damage (SLD) dominates the effectiveness in the high dose region (Barendsen, 1990, 1994, 1997).

The linear-quadratic model is based on well accepted biophysical concepts, involving the assumption that lethal damage can be induced by single-particle tracks and by interaction of damage from multiple particles. It has been found to describe the low-dose region of the survival curves up to 6 Gy rather accurately. Furthermore the LQ-model has been shown to describe adequately dose fractionation effects for normal tissue tolerance and for experimental tumours. The LQ-model has also the advantage that it requires only two parameters to describe radiation dose-survival curves. It allows the separate analysis of changes in effectiveness in the low dose range, mainly determined by the linear term and in the high dose range determined mainly by the quadratic term (Barendsen, 1982; Joiner &

* Suzanne Hovingh¹, Arlene Oei¹, Paul Cobussen¹, Judith W. J. Bergs³, Chris van Bree¹, Hans Rodermond¹, Lukas Stalpers¹, Petra Kok¹, Gerrit W. Barendsen^{1,2} and Johannes Crezee¹
Academic Medical Centre, University of Amsterdam,

¹*Laboratory for Experimental Oncology and Radiobiology (LEXOR),*

Centre for Experimental Molecular Medicine, Department of Radiation Oncology, The Netherlands

²*Department of Cell Biology and Histology, Amsterdam, The Netherlands*

³*Institute of Molecular Biology and Tumor Research, Philipps-University Marburg, Emil-Mannkopff-Str. 2, Marburg, Germany*

Kogel, 2009). An additional advantage of the LQ model is that its parameters can be discussed in terms of specific mechanisms of cell inactivation by radiation (Barendsen, 1990, 1994). Linear-quadratic analyses of hyperthermia- or chemotherapy-induced radiation sensitisation have been reported for exponentially growing and plateau phase human tumour cells in culture and for different experimental rodent cell lines (Franken et al., 1997a, 1997b; van Bree et al., 1997, 2000; Castro Kreder et al., 2004; Bergs et al., 2006, 2007abc). When the additional treatment results in increases of the value of the α -parameter, this indicates that this treatment radiosensitizes at clinically relevant doses. When the additional treatment influences the value of the β -parameter it indicates that the additional treatment has an effect on the repair of sublethal damage.

In order to determine the linear and quadratic parameters and the effects of the different agents on these parameters clonogenic assays (Franken et al., 2006) were conducted of cells after ionizing radiation only and after combined hyperthermia or chemotherapy with radiation treatment. Radiation dose survival curves have been obtained and analysed according to the LQ formula: $S(D)/S(0) = \exp(-\alpha D + \beta D^2)$. (Barendsen, 1982; Barendsen et al., 2001; Franken et al., 2004, 2006). The effects of the different agents on the linear parameter, α and quadratic parameter, β will be described. If significant changes are derived, the values of α/β ratio's are also of interest, because these ratio's show whether the influence of the dose per fraction, dose fractionation and dose rate in radiation treatments is larger or smaller due to the combined treatments. These ratio's may increase with increasing α or decreasing β . A change in the α/β ratio might be clinically of interest for the selection of fractionation schedules.

2. Hyperthermia

Hyperthermia refers to heat treatments of cells or malignancies in which the temperature is elevated in the range of 39°C to 45°C. It is used in combination with chemo- and/or radiotherapy since it has been shown to enhance the anti-cancer effects of both therapies (Gonzalez Gonzalez et al., 1995; Van Der Zee et al., 2002; Crezee et al. 2009). Many *in vitro* studies on the combination of hyperthermia and radiation have shown a synergistic interaction between the two modalities, especially at higher temperatures (above 42°C) (Dewey et al., 1978; Roti Roti, 2004; Raaphorst et al., 1991). This interaction is believed to result from inhibition of repair of radiation-induced DNA damage by hyperthermia (Kampinga et al., 2001; Hildebrandt et al., 2002). The sequence of combined radiation and hyperthermia treatment is important. Optimal sensitization is obtained when radiation and hyperthermia are applied simultaneously or with a short interval (Hall & Giaccio, 2006). In the clinic this is not always possible. In our experiments hyperthermia was applied immediately after radiation treatment.

Despite the clinical goal to reach (cytotoxic) temperatures as high as 43 °C, tumour temperature distributions are in practice heterogeneous. In large areas of the tumour temperatures are often lower than 43°C. Nonetheless, good results have been obtained in locally advanced cervical cancers with tumour temperatures below 43 °C (van der Zee et al., 2000). Mild temperatures have more subtle effects than high temperatures, such as tumour-reoxygenation (Dewhirst et al., 2005; Bergs et al., 2007abc). Recently it has been shown that hyperthermia (42 °C for 1h) transiently breaks down the BRCA2 protein (Krawczyk et al., 2011). In this paragraph the effects of hyperthermia treatment for 1h at 41 or 43 °C on the linear quadratic parameters are summarized. Several different cell types have been studied.

2.1 Effect of hyperthermia treatment on radiosensitivity of RKO cells

The RKO cells, derived from human colon cancer, are relatively sensitive to hyperthermia treatment. Hyperthermia treatment for 1h at 43°C decreases the relative survival to less than 0.01 and combination with radiation doses in excess of 5 Gy always resulted in a situation in which no colony formation was observed. Treatment of cells with 41°C hyperthermia (1h) alone had little effect and resulted in a surviving fraction of 0.8 ± 0.4 in immediately plated (ip) cells and of 0.9 ± 0.1 in delayed plated (dp) cells. When cells were treated at 41°C for 1h immediately prior to irradiation, a significant ($p < 0.001$) enhancement of cellular radiosensitivity was observed both in ip (figure 1A) and dp (figure 1B) cells.

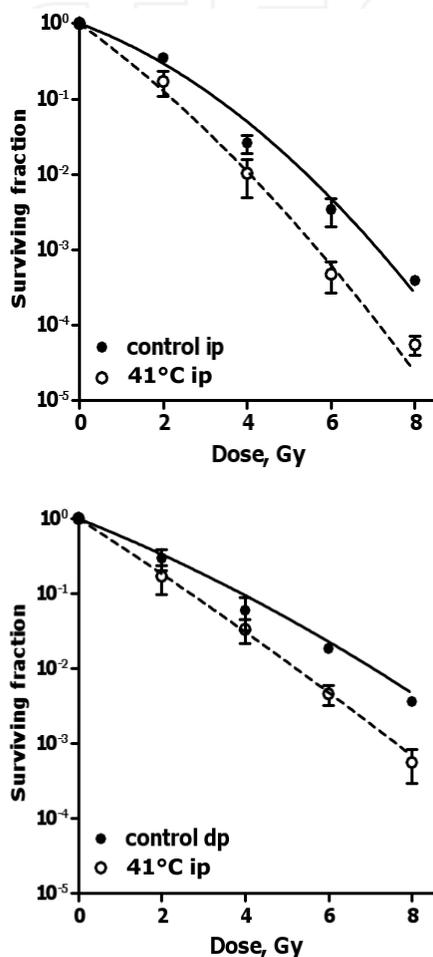


Fig. 1. Radiation survival curves of confluent cultures of RKO cells (human colon cancer cells) plated immediately after irradiation, ip (top) or 24h after irradiation, dp (below) with or without hyperthermia pre-treatment at 41°C for 1h. Means with standard errors of at least three experiments are shown

The effects of hyperthermia on the LQ parameters are summarized in table 1. The value of the linear parameter α increased by a factor 1.7-1.8 while the value of the β parameter even increased with a factor as high as 2.5-7.0. One must bear in mind that the quadratic component in this cell line is very small and small changes can have a large effect on the numerical values of β .

2.2 Effect of hyperthermia treatment on radiosensitivity of SW-1573 cells

SW-1573 cells are derived from a human lung tumour and are much less sensitive to hyperthermia treatment than RKO cells. Studies were performed to evaluate whether pretreatment with hyperthermia at 41°C or at 43°C in SW-1573 cells was able to enhance the radiosensitivity of these cells. Hyperthermia treatment at 41°C for 1h without radiation did not result in a decrease of the surviving fraction for ip and dp cells as compared to radiation alone. One hour hyperthermia treatment at 43°C decreased survival to 0.5 ± 0.1 for ip and to 0.4 ± 0.2 for dp cells. Pre-treatment of cells at 41°C for 1h did not alter cellular radiosensitivity of both ip and dp cells (figure 2A). However, 1h treatment at 43°C resulted in a significant ($p < 0.001$) radiation enhancement both in ip and dp cells (figure 2B). In table I the values of the linear-quadratic parameters for radiation alone and for combined treatments are given. Hyperthermia treatment for 1 h at 41°C did result in an increase of the value of β by a factor 1.3-1.8 while the value of α even decreased. Hyperthermia treatment for 1 h at 43°C result in an increase of the value of α by a factor 2.3-4.4 while the value β increased with a factor 1.8-2.0.

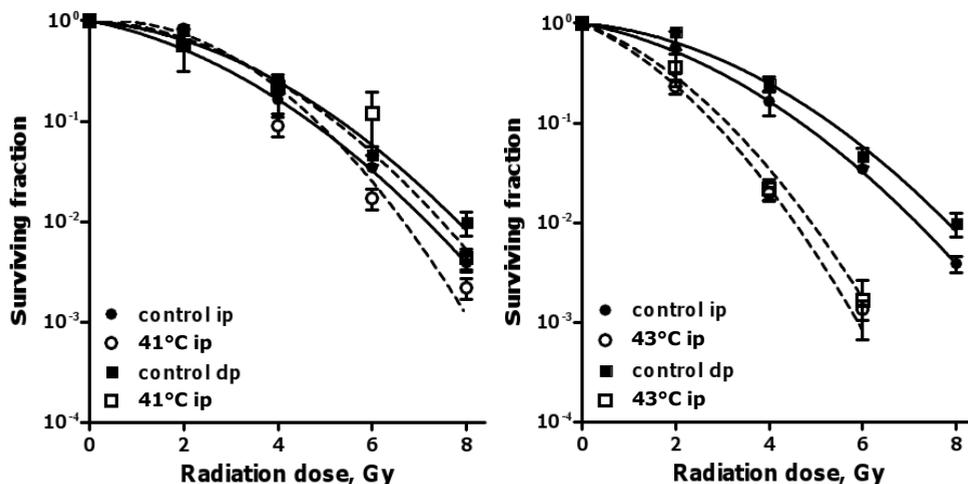


Fig. 2. Radiation survival curves of confluent cultures of SW-1573 cells (human lung tumour cells) plated immediately after irradiation (ip) or 24h after irradiation (dp) with or without hyperthermia pre-treatment at 41°C (left) or at 43°C (right) for 1h. Means with standard errors of at least three experiments are shown.

Cells	Treatment	α (Gy ⁻¹)	β (Gy ⁻²)	α/β	α -enhanc factor	β -enhanc factor
RKO ip	sham	0.55 ± 0.09	0.02 ± 0.01	27.5 ± 14.1		
	HT 41 1h	0.93 ± 0.09*	0.05 ± 0.02	18.6 ± 7.7	1.7 ± 0.3	2.5 ± 1.6
RKO dp	sham	0.47 ± 0.09	0.01 ± 0.01	47.0 ± 47.6 ¹		
	HT 41 1h	0.83 ± 0.08*	0.07 ± 0.02	11.9 ± 3.6	1.8 ± 0.4	7.0 ± 7.3
SW1573 ip	sham	0.21 ± 0.02	0.06 ± 0.02	3.5 ± 1.2		
	HT 41 1h	0.06 ± 0.02	0.11 ± 0.03	0.6 ± 0.2	0.3 ± 0.1	1.8 ± 0.8
	HT 43 1h	0.49 ± 0.04*	0.12 ± 0.03	4.1 ± 1.1	2.3 ± 0.3	2.0 ± 0.8
SW1573 dp	sham	0.09 ± 0.02	0.06 ± 0.02	1.5 ± 1.6		
	HT 41 1h	0.05 ± 0.02	0.08 ± 0.02	0.6 ± 0.6	0.6 ± 0.3	1.3 ± 0.6
	HT 43 1h	0.40 ± 0.04*	0.11 ± 0.03	3.6 ± 1.1	4.4 ± 1.1	1.8 ± 0.8

Sham= is radiation only; ip=immediately plated; dp=delayed plated. * Significant from sham p<0.05.

¹The α/β has a large variation because of the high uncertainty of the value of β .

Table 1. Values of the linear-quadratic parameters α and β , α/β and enhancement factors from cells treated with ionizing radiation only and after combined radiation and hyperthermia treatment

3. Cisplatin

Cisplatin is a widely used anti-cancer drug, often combined with radiotherapy (Gorodetsky et al., 1998). Chemo-radiation application based on cisplatin has now become the standard treatment for, among others, locally advanced cervical carcinoma (Duenas-Gonzalez et al., 2003) and locally advanced non-small cell lung cancer (NSCLC) (Loprevite et al., 2001). There have been many studies on the radiation sensitizing effect of cisplatin, but results vary from a clear cisplatin-induced radiosensitization (Begg et al., 1986; Nakamoto et al., 1996; Bergs et al., 2006, 2007ab) to only an additive effect on cell survival (Fehlauer et al., 2000). Cisplatin and radiation have in common that their cellular target is DNA (Rabik & Dolan, 2007).

Cisplatin causes DNA damage by the formation of inter- and intrastrand adducts (Crul et al., 2002). The cisplatin-DNA adducts can cause cell cycle arrest, inhibition of DNA replication and transcription, and eventually apoptosis (Myint et al., 2002). Repair inhibition of DNA has also been implicated (Lawrence et al., 2003). The most important repair pathways reported to be involved in cisplatin-induced DNA damage repair are nucleotide excision repair (NER) and/or homologous recombination (HR) (Haveman et al., 2004; De Silva et al., 2002). An additional route for the repair of cisplatin-DNA interstrand adducts is the post-replication/translation repair pathway which helps the cell to tolerate or bypass the lesion (Dronkert & Kanaar, 2001). Irradiation causes repairable (potentially lethal) and non-repairable (lethal) lesions to the DNA which are induced independently. The ultimate effect of the repairable lesions depends on competing processes of repair and misrepair. The repair of the potentially lethal damage (PLDR) is reflected by the difference in survival between immediately and delayed plated cells. Inhibition of PLDR is implicated to play a role in cisplatin-induced radiation sensitization (Bergs et al., 2006). More specifically, cisplatin-induced radiation sensitization has been shown to occur through inhibition of the

non-homologous end joining (NHEJ) pathway and recombinational repair (Myint et al., 2002; Haveman et al., 2004; Dolling et al., 1999).

In this paragraph the radiation sensitization of cisplatin on the lung tumour cell line SW1573 and the cervical tumour cell line Siha is described as changes in linear and quadratic parameters. In figure 3 survival curves are shown for SW1573 lung tumour cells after radiation alone and after radiation combined with cisplatin treatment ($1 \mu\text{M}$ for 1 h). Cisplatin was added to the cultures just before radiation. The survival curves are obtained directly (ip) and 24 h after (dp) treatment to determine potentially lethal damage repair. A slight, but statistically significant effect of cisplatin on the radiosensitivity was only

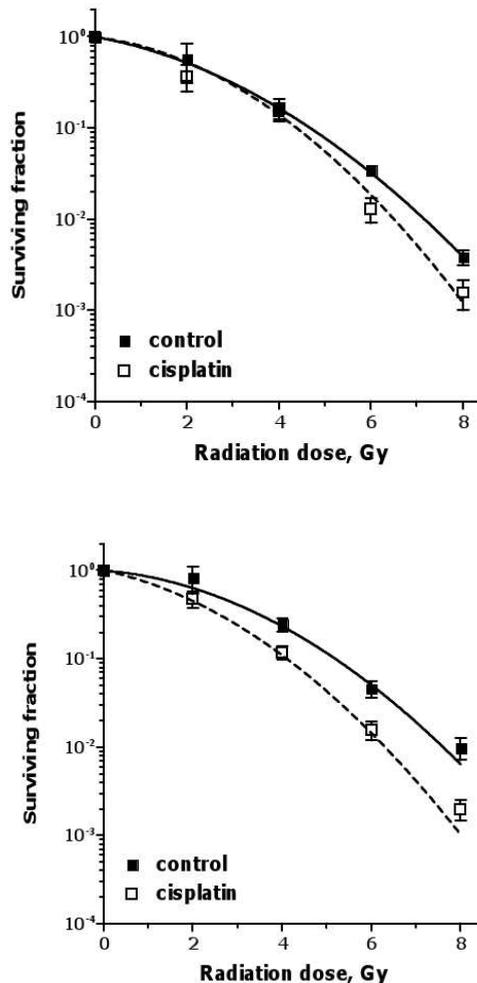


Fig. 3. Radiation survival curves of confluent cultures of SW-1573 cells plated immediately after irradiation, ip (top) or 24 h after irradiation, dp (below) with or without $1 \mu\text{M}$ cisplatin treatment for one hour. Means with standard errors of at least three experiments are shown.

observed in delayed plated cells ($p = 0.02$). This was also described by an increase in the α - and β -value (table 2). Only for the delayed plated cells an increase with a factor of 2.5 for the value of α was obtained by cisplatin treatment. For both plating conditions an increase with a factor of 1.2 was obtained for the value of β . In the table 2 also the effects on the linear and quadratic parameters of different plating conditions are presented as well as a 1 h incubation with 1 or 5 μM cisplatin and a continuous incubation with cisplatin during the complete duration of the clonogenic assay. It is obvious that the cervical tumour cells Siha are more radiosensitized with 1 μM continuous cisplatin incubation than the SW1573 lung tumour cells.

Cells	Treatment	α (Gy ⁻¹)	β (Gy ⁻²)	α/β	α -enhanc factor	β -enhanc factor
SW1573 ip	sham	0.21 ± 0.09	0.061 ± 0.016	3.4 ± 1.7		
	1 μM cisplatin (1h)	0.21 ± 0.08	0.072 ± 0.018	2.9 ± 1.3	1.0 ± 0.6	1.2 ± 0.4
SW1573 dp	sham	0.10 ± 0.09	0.063 ± 0.016	1.6 ± 1.5		
	1 μM cisplatin (1h)	0.25 ± 0.09*	0.077 ± 0.017	3.3 ± 1.4	2.5 ± 2.4	1.2 ± 0.4
SW1573 ppi	sham	0.37 ± 0.12	0.014 ± 0.034	26.4 ± 64.8 ¹		
	1 μM cisplatin (cont)	0.41 ± 0.08	0.019 ± 0.025	21.6 ± 28.7 ¹	1.1 ± 0.4	1.4 ± 3.8
	5 μM cisplatin (cont)	0.58 ± 0.20*	0.030 ± 0.008*	19.3 ± 8.4	1.6 ± 0.7	2.1 ± 5.2
Siha ppi	sham	0.41 ± 0.04	0.01 ± 0.01	41.0 ± 41.2 ¹		
	1 μM cisplatin (cont)	0.81 ± 0.12*	0.02 ± 0.02	40.5 ± 41.0 ¹	2.0 ± 0.4	2.0 ± 2.8

Sham is radiation only; ip=immediately plated; dp=delayed plated; ppi=plated prior to irradiation. * Significant from sham $p < 0.05$. α/β values show that with SW1573 cells the quadratic term is affected more than the linear term, while with Siha cells only the linear term is significantly increased. ¹ The α/β has a large variation because of the high uncertainty of the value of β .

Table 2. Values of the linear-quadratic parameters α and β and enhancement factors from SW1573 and Siha cells treated with ionizing radiation only and after combined radiation cisplatin (1 μM for 1h; 1 μM continuously; 5 μM continuously) treatment

4. Gemcitabine

Gemcitabine (dFdC, Difluorodeoxycytidine) is a deoxycytidine analogue with clinical activity in non-small cell lung cancer (NSCLC) and pancreatic cancer (Fosella et al., 1997; Manegold et al., 2000; Castro Kreder et al., 2004). It requires phosphorylation to its active metabolites, gemcitabine-diphosphate (dF-dCTP) and gemcitabine-triphosphate (dF-dCTP), with the initial phosphorylation by deoxycytidine kinase (dCK) being the rate limiting step (Heinemann et al., 1992; Shewach et al., 1994). The dF-dCTP inhibits ribonucleotide reductase which regulates the production of deoxynucleotides necessary for DNA synthesis and repair (Plukett et al., 1995). The depletion of the deoxynucleotides leads to an increased

incorporation of the dF-dCTP into DNA, blocking DNA synthesis (masked chain termination). After incorporation of the dF-dCTP into the DNA an increase in the number of DNA single-strand breaks, chromosome breaks and micronuclei has been observed (Auer et al., 1997).

Both in vitro and in vivo studies have shown that gemcitabine is a potent radiosensitizer (Shewach et al., 1994; Rockwell & Grindey, 1992; Lawrence et al., 1996; Latz et al., 1998; Gregoire et al. 1998; Milas et al., 1999; van Putten et al., 2001, Wachters et al., 2003, Castro Kreder et al., 2003). However, in an early study in non-small cell lung cancer patients, concurrent gemcitabine and radiotherapy resulted in unacceptable pulmonary toxicity related to the large volume of radiation delivered to the lung (Scalliet et al., 1998). More recent ongoing phase I trials show that concurrent gemcitabine at lower doses and radiotherapy is feasible without severe pulmonary toxicity (Manegold et al., 2000; Blackstock et al., 2001). Its unique mechanism of action, its lack of overlapping toxicity and its favourable toxicity profile define gemcitabine as an ideal candidate for combination therapy (Manegold et al., 2000). Currently many randomized studies are ongoing in which gemcitabine is combined with radiotherapy.

Gemcitabine radiosensitization is studied in a gemcitabine sensitive and resistant human lung tumour cells, SWp and SWg, resp., and in gemcitabine sensitive and resistant human ovarian tumour cells, A2780 and AG6000, resp. (Van Bree et al., 2002; Bergman et al., 2000). Gemcitabine was given 24 h before radiation treatment. The SWp is in fact similar to the SW1573 cell line which has been described above. It is called here SWp to distinguish it from SWg, the gemcitabine resistant counterpart which has been developed by van Bree et al. (2002). The lung tumour cells have different sensitivities to radiation alone as compared to the ovarian cancer cells (Van Bree et al., 2002; Bergman et al., 2000).

In table 3 the linear and quadratic parameters of the different cell lines obtained after analyses of the radiation dose survival curves for radiation alone and after combined radiation and gemcitabine treatment are summarized. SWp and SWg were almost equally sensitive to ionizing radiation alone with respect to the low dose region described by the α -value of the linear quadratic formula (Table 3). A slight increase in survival was observed in SWg cells in the high dose region which was reflected by a slightly lower β -value of the linear-quadratic formula (0.040 ± 0.006 vs 0.055 ± 0.008). The human ovarian carcinoma cell line A2780 and its gemcitabine-resistant variant AG6000 were equally sensitive to ionizing radiation. The surviving fractions of the different cell lines after incubation with gemcitabine alone are: SWp 10 nM: 0.52 ± 0.06 ; SWg 10 μ M: 0.95 ± 0.03 , 100 μ M: 0.24 ± 0.11 ; A2780 2 nM: 0.82 ± 0.08 , 10 nM: 0.21 ± 0.08 ; AG6000 20 μ M: 0.62 ± 0.07 , 50 μ M: 0.22 ± 0.04 .

As can be observed in figure 4 and table 3 radiosensitization is observed with gemcitabine-sensitive as well as in gemcitabine resistant cells. For the resistant cells much higher gemcitabine doses are needed for the radiation sensitization to result in similar cytotoxicity. Both gemcitabine-sensitive cell lines SWp and A2780 are sensitized by incubation with 10 nM of gemcitabine for 24 h before irradiation while the SWg and AG6000 are not sensitized with this dose of gemcitabine. The sensitization is described by an increase in the α -values with factors of 3 and 1.4 respectively, whereas the β -values are not significantly altered. Higher concentrations of Gemcitabine (50 and 100 nM resp.) are required to sensitize gemcitabine-resistant AG6000 and SWg cells to irradiation. For the SWg cells, the radiosensitization was reflected by an increase by a factor of 2.25 in the value of β , whereas in the AG6000 only the α -value was increased by factor of 1.3.

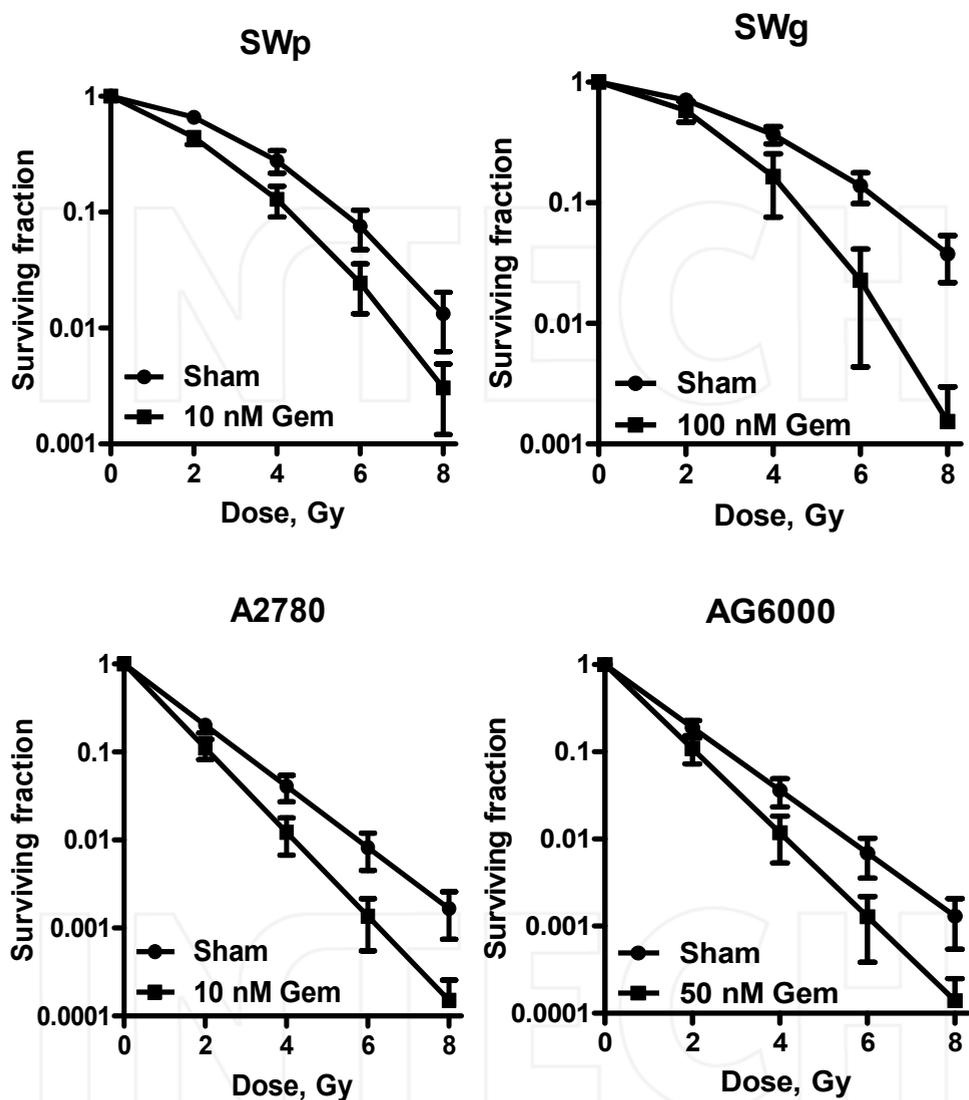


Fig. 4. Radiation sensitization after 24 h incubation with different concentrations of gemcitabine in gemcitabine sensitive SWp and resistant SWg lung tumour cells and in gemcitabine sensitive A2780 and resistant AG6000 ovary cancer cells. Surviving fractions are corrected for gemcitabine toxicity alone (for values see text). Cells are plated immediately after irradiation. Means with SEM of at least three separate experiments are shown.

Cells	Treatment	α (Gy ⁻¹)	β (Gy ⁻²)	α/β	α -enhanc factor	β -enhanc factor
SWp	sham	0.10 ± 0.03	0.055 ± 0.008	1.8 ± 0.6		
	10nM gemcitabine	0.30 ± 0.06*	0.053 ± 0.007	5.7 ± 1.4	3.0 ± 2.8	0.96 ± 0.2
SWg	sham	0.09 ± 0.02	0.040 ± 0.006	2.3 ± 0.6		
	100 μM gemcitabine	0.09 ± 0.03	0.090 ± 0.041†	1.0 ± 0.6	1.0 ± 0.5	2.3 ± 1.1
A2780	sham	0.80 ± 0.10	na			
	10nM gemcitabine	1.10 ± 0.15*	na		1.4 ± 0.3	
AG6000	sham	0.83 ± 0.13	na			
	50μM gemcitabine	1.11 ± 0.20†	na		1.3 ± 0.3	

significant difference with *P<0.01, †P<0.05, na is not applicable

Table 3. Values of the linear-quadratic parameters α and β and enhancement factors from cells treated with ionizing radiation only and gemcitabine-sensitized radiation dose survival curves of gemcitabine-sensitive (SWp and A2780) and gemcitabine-resistant (SWg and AG6000) cells.

5. Halogenated pyrimidines

Incorporation of halogenated pyrimidines (HPs), chloro-, bromo- and iodo-deoxyuridine (CldUrd, BrdUrd, IdUrd) into DNA is known to sensitise cells to ionizing radiation (Franken et al. 1997ab, 1999ab; van Bree et al. 1997; Iliakis et al. 1999; Miller et al. 1992ab). The induced radiosensitisation increases with the degree of thymidine-replacement. The mechanism of radiation sensitisation by the HPs has been suggested to be either an increase in the amount of DNA damage induced by radiation, an influence on repair of sublethal damage (SLD), and/or an enhanced expression of potentially lethal damage (PLD) (Jones et al. 1995; Franken et al. 1997). Since different processes are involved in these phenomena several mechanisms might contribute to the radiosensitisation.

HPs have been suggested to provide an advantage in radiotherapy as radiosensitisers of cells in rapidly growing tumours, in particular in clinical conditions in which critical normal tissues show limited proliferation and as a consequence take up less HP. Labelling depends on the growth fraction, cell loss, cell cycle time and potential doubling time. Of special importance for sensitisation is the rate at which non-cycling cells are recruited into the proliferative compartment during exposure to HPs and a course of radiotherapy. However, even in rapidly growing tumours, cells may, after proliferative cycles, move into a non-proliferative stage. This might compromise the degree of radiation sensitisation if resting cells are less affected by HPs, or are better able to cope with additional damage by repair of PLD.

Here the results of radiosensitization after incubation with 4 μM IdUrd for 72 h are presented. IdUrd-induced radio sensitisation was obtained in all studied cell lines, SW1573, RUCII (Rat urether carcinoma), R1 (Rat rhabdomyosarcoma) and V79, in exponentially growing and in plateau-phase cells. Values of α and β derived by linear-quadratic analyses

of survival curves of exponentially growing cells and plateau-phase cells are presented in table 4. Survival curves of SW cells and V79 cells are given in figure 5. The plating conditions of the V79 cells, i.e. exponentially growing cells plating before or after irradiation (ppi or dp resp.), and plateau phase cells plated immediately or 6-24 h delayed after irradiation (ip or dp resp.) had no influence on the factor of increase of the α -value. It is shown that the value of the linear parameter, α can be enhanced by a factor of 1.9 to 7.5 and that in general low values of α are enhanced more than higher values of α . The value of β is less enhanced and the enhancement factor ranges from 0.7 to 2.4.

The direct comparison between immediate and delayed plating of plateau-phase cells and between plateau phase and exponentially growing cells shows significant quantitative differences. The data on the linear and quadratic parameters described here provide various new insights in the interpretation of radiosensitisation of delay plated plateau-phase cells. It is demonstrated that in delay plated HP-sensitized plateau phase cells PLD is not abolished.

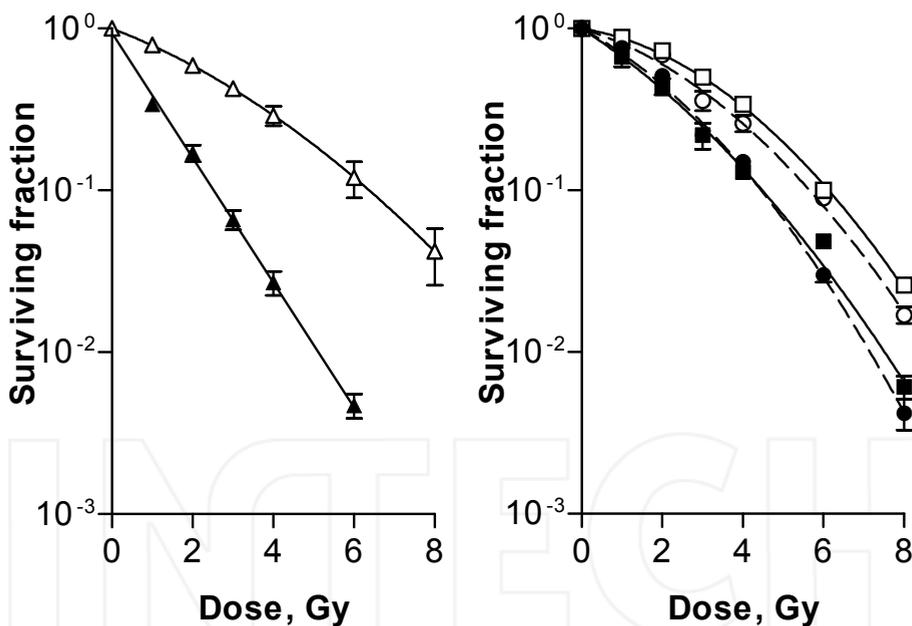


Fig. 5. Radiation dose-survival curves of exponentially growing SW-1573 cells (left) without IdUrd (open triangles) and after incubation with 4 μ M IdUrd (closed triangles) and plateau-phase cells (right) plated immediately after irradiation (dashed lines) and plated 24 h after irradiation (solid lines) without IdUrd (open symbols) and after incubation with 4 μ M IdUrd (closed symbols). Each point represents the mean value of 3 different experiments \pm sem.

Cell line	α (Gy ⁻¹) control	β (Gy ⁻²) control	α (Gy ⁻¹) IdUrd- sens	β (Gy ⁻²) IdUrd- sens	α/β control	α/β IdUrd- sens	α - enhanc factor	β - enhanc factor
SW 1573 cells Exp growing ip	0.22 ± 0.01	0.022 ± 0.001	0.83 ± 0.06	na	10.0 ± 0.6	na	3.8 ± 0.3	na
SW 1573 cells Plateau phase ip	0.17 ± 0.03	0.042 ± 0.004	0.31 ± 0.03	0.047 ± 0.005	4.1 ± 0.8	6.6 ± 1.0	1.8 ± 0.4	1.1 ± 0.2
SW 1573 cells Plateau phase dp	0.09 ± 0.02	0.046 ± 0.002	0.37 ± 0.04	0.033 ± 0.006	2.0 ± 0.4	11.2 ± 2.4	4.1 ± 1.0	0.7 ± 0.1
RUCII cells Exp growing ppi	0.008 ± 0.007	0.025 ± 0.001	0.06 ± 0.02	0.026 ± 0.001	0.3 ± 0.3	2.3 ± 0.8	7.5 ± 7.0	1.0 ± 0.1
R1 cells Exp growing ppi	0.23 ± 0.01	0.068 ± 0.003	0.44 ± 0.05	0.075 ± 0.016	3.4 ± 0.2	5.9 ± 1.4	1.9 ± 0.3	1.1 ± 0.2
V79 cells Exp growing ip	0.18 ± 0.02	0.017 ± 0.003	0.38 ± 0.04	0.023 ± 0.007	10.6 ± 2.2	16.5 ± 5.3	2.1 ± 0.3	1.4 ± 0.5
V79 cells Exp growing ppi	0.15 ± 0.02	0.013 ± 0.003	0.29 ± 0.03	0.016 ± 0.004	11.5 ± 3.1	18.1 ± 4.9	1.9 ± 0.3	1.2 ± 0.4
V 79 cells Plateau phase ip	0.09 ± 0.03	0.026 ± 0.004	0.17 ± 0.02	0.062 ± 0.005	3.5 ± 1.3	2.7 ± 0.4	1.9 ± 0.7	2.4 ± 0.4
V 79 cells Plateau phase dp	0.07 ± 0.02	0.020 ± 0.002	0.30 ± 0.03	0.024 ± 0.004	3.5 ± 1.1	12.5 ± 2.4	4.3 ± 1.3	1.2 ± 0.2

Means with SEM of at least three separate experiments are shown. ip=immediately plated after irradiation; dp=delayed plated after irradiation; ppi=plated prior to irradiation; na=not applicable.

Table 4. Values of the linear-quadratic parameters α and β and enhancement factors of several cell lines treated with ionizing radiation only and after sensitization with iododeoxyuridine (incubation with 4 μ M IdUrd for 72 h)

6. PARP-1 inhibitors

The effect of the Parp-1 inhibitor NU-1025 on the linear and quadratic parameters was tested in Mouse embryonic fibroblasts. Parp1 also known as Poly (ADP-ribose) polymerase is an enzyme which is involved in the single strand break (SSB) repair of the DNA. The DNA SSB induced by ionizing radiation are mostly repaired by the base excision repair system, BER, whereas the DNA DSB are repaired by non homologous endjoining NHEJ or homologous recombination, HR. Inhibiting Parp-1 activity reduces the single strand break repair (Bouchard, 2003). Besides its role in BER Parp-1 is involved in many nuclear processes like DNA replication, transcription, DSB repair, apoptosis and genome stability (Rouleau et al. 2010; Bouchard et al. 2003, Löser et al. 2010). Recently it was hypothesized that cells deficient in BRCA2 or BRCA1 are particularly sensitive to inhibition of Parp-1 (Rouleau et al. 2010, Krawczyk et al. 2011). During DNA replication SSBs are induced. In the absence of Parp-1 these SSB are transformed in DSB. These DSB are repaired with homologous recombination (HR). Therefore cells deficient in HR (e.g. BRCA1 or BRCA2 tumours) might be sensitive to Parp-1 inhibitors. As Parp-1 is involved in many DNA repair processes, Parp-1 inhibitors might work well as radiosensitizers (Löser et al. 2010). As can be observed in figure 6 a modest sensitization effect by the Parp-1 inhibitor NU-1025 was obtained and increase of the value of α in the repair deficient cell line was larger than in the repair proficient cell line, 1.4 vs 1.2 respectively (table 5). The radiation dose survival curves of these MEF cells did not show a shoulder and therefore the quadratic parameter, β , could not be determined.

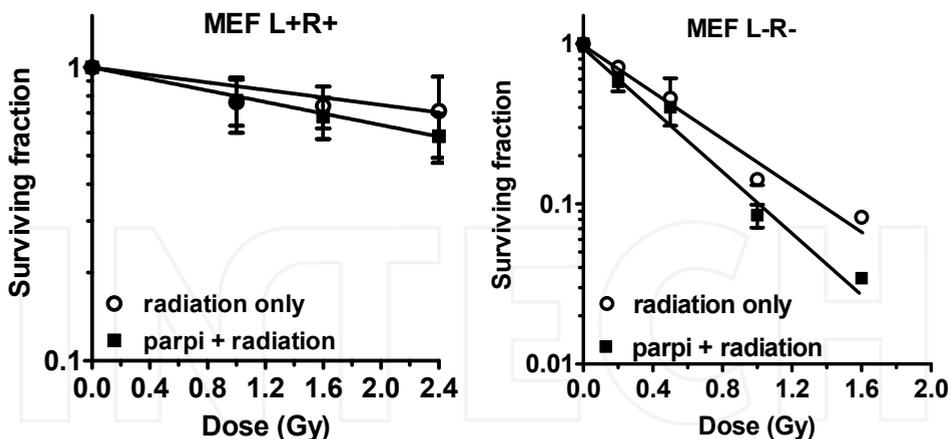


Fig. 6. Radiation dose-survival curves of Mouse embryonic fibroblasts (A: LigIV+/+, Rad54+/+) A and LigaseIV deficient and RAD54 deficient (B: LigIV-/-, Rad54-/-) mouse embryonic fibroblasts. Open circles radiation only curves, closed squares: radiation + Parp inhibitor. Cells were treated with 100 μ M Nu-1025 for 24 h before irradiation.

MEF Cells	Treatment with Parp-i	α (Gy ⁻¹)	β (Gy ⁻²)	α -enhanc. factor
LigIV+/+,Rad54+/+	No	0.28 ± 0.01	na	
LigIV+/+,Rad54+/+	yes	0.33 ± 0.03	na	1.2
LigIV-/-,Rad54-/-	no	1.59 ± 0.18	na	
LigIV-/-,Rad54-/-	yes	2.28 ± 0.42	na	1.4

Na= not applicable.

Table 5. Values of the linear parameter, α , and the enhancement factors. The quadratic parameter β could not be determined in these MEF cells.

7. Discussion and conclusion

In most cases an increase of the α -component was observed which corresponds to an enhanced (potentially) direct lethal damage (PLD) at low doses. The β -component, which is assumed to depend on the interaction of sublethal lesions (SLD), was rarely affected by the studied radiosensitization agents. Moreover, it appeared that more radioresistant cell lines were more sensitised than the radiosensitive lines. Furthermore it can be concluded that radiosensitization is also dependent on cell cycle stage like plateau or exponentially growing phase or post treatment plating conditions.

Hyperthermia is an excellent radiosensitizer which can already be effective at mild temperatures. One hour hyperthermia treatment at 41°C without radiation had only a small cytotoxic effect in both the heat sensitive and the heat resistant cell line. This is in agreement with the general idea of cell kill induction at temperatures $\geq 42^\circ\text{C}$ for 1h or more (Dewhirst, 2005). Hyperthermia treatment at 43°C for 1h did not have a large cytotoxic effect in heat resistant SW-1573 cells. Radiosensitization by 41°C temperature hyperthermia was observed in RKO, but not in SW-1573 cells. The ability of mild temperatures (in the range of 40-42°C) hyperthermia to increase radiosensitivity of human tumor cells has been shown to be cell line dependent (Ruy et al., 1996; Franken et al., 2001; Bergs et al., 2007ab; Larsson & Ng, 2003; Murthy et al., 1977). In a study by Xu et al. (1999) 41.1°C pre-treatment of cells for 1h did not induce radiosensitization whereas treatment for 2h or more resulted in radiosensitization, in the hyperthermia resistant, but not in the hyperthermia sensitive cell line (Xu et al., 1999). However, simultaneous treatment of the sensitive cell line with 1h 41.1°C hyperthermia and radiation did increase cellular radiosensitivity (Xu et al., 2002). An important mechanism of mild hyperthermia induced radiosensitization *in vivo* is the reoxygenation of tumors by an increase in blood flow (Vujaskovic et al., 2004; Oleson & Robertson, 1995; Song et al., 1995). Recently it was demonstrated that the BRCA-2 protein is transiently inhibited by mild hyperthermia (Krawczyk et al., 2011). Also translocation of the Mre11 DSB repair protein from the nucleus to the cytoplasm has been implicated (Xu et al., 2002, 2007). However, disappearance of Mre11 protein foci at the sites of irradiation induced DNA double strand breaks by 41°C pre-incubation of cells was not observed (Krawczyk et al., 2011; Bergs, 2007a). A role for mitotic catastrophe occurring as a result of G2/M checkpoint abrogation has also been suggested (Mackey & Ianzini, 2000). It has been shown that radiosensitization by 41-43°C hyperthermia correlates with an increased number of chromosomal fragments, but not of color junctions, at 24h after treatment compared to radiation alone (Bergs et al., 2008).

It is shown that cisplatin causes radiosensitization as measured by clonogenic survival, but only after allowing a potentially lethal damage repair (PLDR) time of 24 hours. These results are in agreement with those of Wilkins et al. (1993) who investigated the effect of cisplatin and radiation on PLDR in confluent cultures of two different brain tumor cell lines. Wilkins et al. (1993) also observed no radiosensitization by cisplatin in immediately plated cells whereas a cisplatin-induced radiosensitization was seen in cells plated eight hours after irradiation. Their results indicate that the radiosensitizing effect of cisplatin occurs through the inhibition of post-irradiation recovery. The strongest inhibition of PLDR was achieved when cisplatin was administered shortly before or after irradiation (Wilkins et al., 1993). In our experiments, cells were irradiated while cisplatin was present in the medium.

Results from studies using exponentially growing cell cultures vary from a cisplatin-induced radiosensitization (Loprevite et al., 2001; Begg et al. 1986; Nakamoto et al. 1996; Huang et al. 2004) to only an additive effect (Gorodetsky et al., 1998; Loprevite et al., 2001; Britten et al., 1996; Monk et al., 2002). The effect of cisplatin treatment on radiosensitivity may depend on the cell type used. Loprevite et al (2001) observed synergism in a squamous lung carcinoma cell line when exposed to cisplatin, whereas an adenocarcinoma of the lung was not sensitized by cisplatin. Even cell lines derived from a single biopsy can differ in the response to cisplatin and radiation combination therapy (Britten et al., 1996).

Although dependence on cell cycle phase (Meyn et al., 1980; Krishnaswamy & Dewey, 1993), cisplatin incubation time and the sequence of treatment modalities have been implicated (Gorodetsky et al., 1998; Meyn et al., 1980; Krishnaswamy & Dewey, 1993), there is currently no consensus to account for the varying response of cells to cisplatin and radiation.

The mechanism of cisplatin induced radiosensitization might be due to the inhibition of the DNA repair, NHEJ and HR, pathways (Myint et al., 2002; Dolling et al., 1998). The Ku protein complex, which plays an important role in NHEJ, was demonstrated to show a reduced ability to translocate on DNA containing cisplatin-DNA adducts compared with undamaged DNA. This resulted in a decreased interaction between Ku and DNA-PK_{cs} (Turchi et al., 2000) However, the biochemical processes that cisplatin undergoes in the cell are complex and the intracellular fate of cisplatin may be linked to copper transport (Muffia & Fojo, 2004). Therefore, other processes such as the formation of peroxy complexes inside the cell might be involved in cisplatin-induced radiosensitization (Dewit, 1987). Bergs et al. (2006) demonstrated an increase in the induction of apoptosis after combined treatment as compared to radiation or cisplatin alone at 24 h after treatment. This was confirmed in several other studies (Kumala et al., 2003; Guchelaar et al. (1998). These apoptotic effects observed by Bergs et al. (2006) correlated with clonogenic survival. Fujita et al. (2000) also observed an inhibitory effect of the combination of cisplatin and radiation on the survival of lung tumor cells and ascribed this effect on the induction of tumor cell apoptosis.

In conclusion, a radiosensitizing effect of cisplatin on cell survival is observed in confluent cultures when cells were replated after a 24 hour incubation period during which PLD repair could take place. In contrast, cisplatin did not induce a significant radiosensitization after immediate plating.

Several studies have shown that gemcitabine is a potent sensitizer of ionizing radiation (Shewach et al., 1994; Gregoire et al., 1999; Ostruszka & Shewach, 2000). Among other proposed mechanisms of action, the effect of gemcitabine on cell cycle distributions may be the most important (Milas et al., 1999; Van Putten et al., 2001). In our studies, both

gemcitabine-sensitive cell lines SWp and A2780 could be sensitized to irradiation when cytotoxic gemcitabine-treatments were given. The radiosensitization was accompanied by a clear arrest of cells in early S phase which has been argued to be vital for gemcitabine-induced radiosensitization (Latz et al., 1998). Both cell lines showed an increase in α -value indicating the efficacy of gemcitabine-induced radiosensitization in the clinically relevant dose range. Although the gemcitabine resistant cells still could be sensitized only much higher gemcitabine doses were necessary to reach an effect. In the resistant ovarian carcinoma cell line AG6000 this was demonstrated by an increase in the value of α . In contrast with this change, in the gemcitabine resistant lung tumour cell line an increase in the β -value was obtained, the α -value was not affected. In both gemcitabine-resistant cell lines the sensitivity to ionizing radiation alone was not altered. It is reported that gemcitabine resistant tumours are cross-resistant to related drugs like Ara-c (Ruiz van haperen et al., 1994; Peters et al., 1996). In both gemcitabine-resistant cell line, AG6000 and SWg, this was indeed the case (Van Bree et al., 2002). Moreover, the AG6000 cells were also more resistant to cisplatin and taxoids (Bergman et al., 2000). However, no altered sensitivity was found in SWg cells for cDDP, paclitaxel, MTX and 5 FU, while AG6000 cells were 2.5-fold more sensitive to MTX (Bergman et al., 2000). These findings indicate that patients previously treated with gemcitabine may receive additional radiotherapy with or without cDDP or paclitaxel.

The HP-induced-radiosensitisation is mainly due to an increase in the linear parameter α . The quadratic parameter, β , is only rarely influenced. Different mechanisms involved in the radiosensitisation induced by halogenated pyrimidines have been described. Wang et al. (1994) suggested that in exponentially growing cells increased DNA damage production was the major component of radiosensitisation while in plateau-phase cells radiosensitisation occurred through inhibited repair and/or enhanced fixation of potentially lethal damage. The increase of the α values for exponentially growing cells as found in our study, indicates an increase in the number of directly lethal events due to the HPs. This is in agreement with observations of Webb et al. (1993) and Jones et al. (1995) which suggest that an important mechanism of radiosensitisation involves an increase of effective DNA double strand breaks. Miller et al. (1992ab) have suggested that radiation-induced damage in cells which have HPs incorporated into the DNA after low-LET irradiation resembles the damage produced by high-LET radiation. In plateau-phase cells plated immediately after irradiation the increase of α might be due to the same mechanism as involved in exponentially growing cells. In these cells also an increase of β was observed indicating that accumulation of sublethal lesions contributed significantly (Barendsen 1990). Due to the immediate plating after irradiation this sublethal damage might be fixated.

Greatest increases in α were found in delayed plated plateau-phase cells. This radiosensitisation can be interpreted as an enhanced fixation of potentially lethal damage due to immediate DNA damage and/or to damaged DNA repair function in these cells expressed during the interval before delayed plating. The value of β in these cells returned to values as found in cells not containing HPs. This demonstrates that sublethal damage has been repaired in HP-containing plateau-phase cells.

Because Parp-1 is implicated in several DNA repair processes, Parp-1 inhibitors might be good radiosensitizers. Several studies have already demonstrated the radiosensitizing effect of Parp-1 inhibitors (Albert et al. 2007; Löser et al. 2010; Krawczyk et al. 2011). Loser et al concluded that the effects of Parp-1 inhibitors are more pronounced on rapidly dividing

and/or DNA repair deficient cells. In our study at time of treatment most of the cells in culture had accumulated in G1 phase. Therefore radiosensitization effects are modest. However, the increase of value of the linear parameter, α , of the repair deficient cells was more increased after the parg-1 inhibition than of the repair proficient cells.

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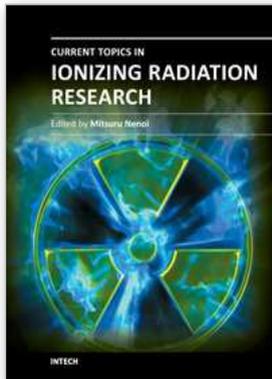
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Since the discovery of X rays by Roentgen in 1895, the ionizing radiation has been extensively utilized in a variety of medical and industrial applications. However people have shortly recognized its harmful aspects through inadvertent uses. Subsequently people experienced nuclear power plant accidents in Chernobyl and Fukushima, which taught us that the risk of ionizing radiation is closely and seriously involved in the modern society. In this circumstance, it becomes increasingly important that more scientists, engineers and students get familiar with ionizing radiation research regardless of the research field they are working. Based on this idea, the book "Current Topics in Ionizing Radiation Research" was designed to overview the recent achievements in ionizing radiation research including biological effects, medical uses and principles of radiation measurement.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821