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Effects of Gemcitabine on Cell Survival and Chromosome Aberrations after Pulsed Low Dose-rate Irradiation

Natasja CASTRO KREDER, Chris VAN BREE, Nicolaas A. P. FRANKEN and Jaap HAVEMAN*

Gemcitabine/X-irradiation/Cell survival/Chromosome/Aberrations/Chromosome/Fragments.

The radiosensitizing potential of gemcitabine (2′,2′-difluoro-2′-deoxycytidine) was studied in combination with pulsed low dose-rate irradiation. The experiments were carried out with a human lung carcinoma cell line SW1573. These were irradiated at pulsed low dose rate (p-LDR); the average dose rate was 1 Gy/h. In the experiments with gemcitabine, this drug was applied for 24 h at a concentration of 10 nM prior to irradiation. The response of the cells to treatment was tested by using the standard clonogenic assay. Next to the cell-killing effects, damage to chromosomes was also assayed by using whole chromosome Fluorescent In Situ Hybridization (FISH). Damage in chromosomes 2 and 18 was visualized by whole chromosome FISH and scored according to the PAINT method. A clear enhancement of the effects of radiation on cell survival was observed by preincubation of the cells with gemcitabine. The enhancement factor obtained from the p-LDR data was 1.7, which is much lower than the enhancement factor of 2.9 at high-dose rate. We did not observe a consistent increase in color junctions concomitant with radiosensitization. In chromosome 2, a small increase, and in chromosome 18, a decrease, in the number of color junctions was observed after radiation combined with gemcitabine compared to irradiation alone. These differences were not statistically significant. However, for the (unstable) acentric chromosome fragments from both chromosomes, significant changes were observed: In the case of chromosome 2, an increase, and in the case of chromosome 18, a decrease. So these results indicate that gemcitabine has no large and consistent effect on the repair of genomic lesions that induce secondary chromosome breaks. Although it is clear that gemcitabine-induced radiosensitization can be expected when it is combined with brachytherapy, as with radiation at a high-dose rate, the mechanism of radio-osensitization is so far not evident, and further experiments will be needed to elucidate this.

INTRODUCTION

The dose rate is a main factor that affects the biological response to radiation, notably in sparsely ionizing types of radiation such as X-rays or γ-rays. A reduction in the dose rate decreases the biological effect because of the repair of sublethal damage that occurs during a long radiation exposure. At a low dose rate (LDR), the characteristic shoulder observed in survival curves after a high-dose rate (HDR) disappears.1) The magnitude of the dose rate effect varies greatly between different cells. The cell cycle effects of radiation may lead to an inverse dose rate effect19). In some cells, proliferation during LDR irradiation further decreases the effects on survival. Mammalian cell survival curves are presently often described by using the linear-quadratic (LQ) formula: S(D)/S(0) = exp(−αD−βD²).3–5) The parameters α (alpha) and β (beta) are assumed to reflect specific mechanisms of cell killing by radiation. The linear term alpha dominates the response at low doses, and the quadratic term beta plays a major role at high doses. Both α and β are influenced by potentially lethal damage (PLD) repair. This indicates that the repair of PLD can reduce lethal lesions as well as sublethal lesions.4) When the dose rate is reduced, this results in a reduction of the alpha and the beta component. Furthermore, when all sublethal damage can be repaired during irradiation, because of the prolonged exposure at low dose rate, only the alpha component determines the survival curve.

Low dose rate irradiation is used in the clinic in the form of brachytherapy, see, e.g., Yamazaki et al.7) During a brachytherapy session, radiation sources are placed in or close to tumors. Because of the well-localized positioning of the sources, an optimal dose distribution and sparing of normal tissue can be obtained. Brachytherapy is used in the treatment of...
of several malignancies including breast, prostate, and cervical cancer.

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine or briefly dFdC) is a relatively new cytotoxic drug with proven activity in a variety of solid tumors including pancreas and non-small-cell lung cancer\(^8\). Gemcitabine is a deoxycytidine analogue, and for its antitumor activity, phosphorylation to an active triphosphate form is required.\(^9\) To allow phosphorylation to take place, it is necessary that cells are incubated some time before combination with any other treatment. For example, Haveman et al.\(^{10}\) used the same cell line as in the present study, and they showed that 24 h after 10 nM gemcitabine, the maximum effect was obtained in combination with hyperthermia. Its working mechanism is multifactorial, including inhibition of DNA synthesis, interference of the DNA replication by incorporation into the DNA, and depletion of the deoxynucleoside pools by inhibition of ribonucleotide reductase.\(^{11–13}\)

Gemcitabine is a potent radiosensitizer both in vivo and in vitro, but the mechanism of radiosensitization is still not entirely clear.\(^{14–18}\) We have previously reported that the radiosensitizing effect of gemcitabine after HDR irradiation is mainly on the alpha component of the radiation survival curves.\(^{19,20}\) This is also evident from other studies reporting radiosensitization after gemcitabine treatment for which we calculated the LQ parameters, alpha and beta, from the survival curves (see Table 1). The results confirm our previous finding that the influence of gemcitabine on survival after HDR-irradiation is mainly on the alpha component. Because survival after low dose rate (LDR) irradiation is mainly governed by the alpha component, we investigated the radiosensitiz-

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell line</th>
<th>Cancer type</th>
<th>(\alpha) G -Gemcitabine</th>
<th>(\beta) G -Gemcitabine</th>
<th>(\alpha) W -Gemcitabine</th>
<th>(\beta) W -Gemcitabine</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawrence et al.(^{15})</td>
<td>HT29</td>
<td>Human colon cancer</td>
<td>(\alpha) 0.15</td>
<td>(\beta) 0.04</td>
<td>(\alpha) 0.40 (10 nM)</td>
<td>(\beta) 0.03</td>
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<tr>
<td></td>
<td>UMSCC-6</td>
<td>Head&amp;Neck squamous cancer</td>
<td>(\alpha) 0.27</td>
<td>(\beta) 0.02</td>
<td>(\alpha) 0.42 (10 nM)</td>
<td>(\beta) 0.03</td>
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<td></td>
<td>A549</td>
<td>Lung cancer</td>
<td>(\alpha) 0.19</td>
<td>(\beta) 0.03</td>
<td>(\alpha) 0.33 (10 nM)</td>
<td>(\beta) 0.03</td>
<td>1.7</td>
</tr>
<tr>
<td>Ostruszka &amp; Shewach (^{16})</td>
<td>U251</td>
<td>Glioblastoma</td>
<td>(\alpha) 0.20</td>
<td>(\beta) 0.05</td>
<td>(\alpha) 0.52 (10 nM)</td>
<td>(\beta) 0.05</td>
<td>2.6</td>
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<tr>
<td></td>
<td>D54</td>
<td>Glioblastoma</td>
<td>(\alpha) 0.15</td>
<td>(\beta) 0.05</td>
<td>(\alpha) 0.26 (80 nM)</td>
<td>(\beta) 0.04</td>
<td>1.7</td>
</tr>
<tr>
<td>Lawrence et al.(^{17})</td>
<td>Panc-1</td>
<td>Pancreatic cancer</td>
<td>(\alpha) 0.16</td>
<td>(\beta) 0.05</td>
<td>(\alpha) 0.22 (30 nM)</td>
<td>(\beta) 0.09</td>
<td>1.4</td>
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<td></td>
<td>BxPC-3</td>
<td>Pancreatic cancer</td>
<td>(\alpha) 0.03</td>
<td>(\beta) 0.043</td>
<td>(\alpha) 0.26 (10 nM)</td>
<td>(\beta) 0.04</td>
<td>1.4</td>
</tr>
<tr>
<td>Van Bree et al.(^{19})</td>
<td>SW-1573</td>
<td>Lung cancer</td>
<td>(\alpha) 0.10</td>
<td>(\beta) 0.055</td>
<td>(\alpha) 0.30 (10 nM)</td>
<td>(\beta) 0.05</td>
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<tr>
<td>Shewach et al.(^{15})</td>
<td>HT-29</td>
<td>Human colon cancer</td>
<td>(\alpha) 0.08</td>
<td>(\beta) 0.045</td>
<td>(\alpha) 0.27 (10 nM)</td>
<td>(\beta) 0.04</td>
<td>3.4</td>
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<tr>
<td>McGinn et al.(^{30})</td>
<td>UMSCC-1</td>
<td>Head&amp;Neck squamous cancer</td>
<td>(\alpha) 0.22</td>
<td>(\beta) 0.03</td>
<td>(\alpha) 0.34 (30 nM)</td>
<td>(\beta) 0.06</td>
<td>1.5</td>
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<tr>
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<td>MCF7-WT</td>
<td>Breast Cancer</td>
<td>(\alpha) 0.03</td>
<td>(\beta) 0.097</td>
<td>(\alpha) 0.21 (10 nM)</td>
<td>(\beta) 0.14</td>
<td>7</td>
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<tr>
<td></td>
<td>MCF-PDR</td>
<td>Breast Cancer (pleiotropic drug-resistant cells)</td>
<td>(\alpha) 0.01</td>
<td>(\beta) 0.053</td>
<td>(\alpha) 0.14 (10 nM)</td>
<td>(\beta) 0.08</td>
<td>14</td>
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<tr>
<td>Lawrence et al.(^{17})</td>
<td>SW620</td>
<td>Colon cancer</td>
<td>(\alpha) 0.14</td>
<td>(\beta) 0.07</td>
<td>(\alpha) 0.44 (10 nM)</td>
<td>(\beta) 0.08</td>
<td>3.1</td>
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</tbody>
</table>

\(\alpha\): cell survival is mainly governed by the alpha component.
\(\beta\): cell survival is mainly governed by the beta component.
EF: Enhancement factor calculated from the \(\alpha\) values. (\(\alpha\) with/\(\alpha\) without dFdC).
ing chromosome of gemcitabine after LDR irradiation.

Chromosome aberrations are often used to monitor radiation-induced damage to cells, under a variety of experimental conditions, see, e.g., Ofuchi et al.\textsuperscript{21} and Ritter et al.\textsuperscript{22} In a recent report by Rosier et al.,\textsuperscript{23} the authors state that radioenhancement by gemcitabine was associated with a high frequency of cells with residual chromosome aberrations, this based on the results with two different cell lines originating from human head and neck squamous carcinoma. They suggest that this is due to an effect of gemcitabine on the repair of genomic lesions inducing secondary chromosome breaks. These observations by Rosier et al.,\textsuperscript{23} are in contrast with our recent results at HDR.\textsuperscript{20} We observed a small but significant reduction in chromosome aberrations after combined gemcitabine and HDR-irradiation. To further investigate this, we also studied the effect of gemcitabine on the induction of color junctions, dicentric rings, and acentric chromosome fragments after p-LDR-irradiation.

**MATERIALS AND METHODS**

**Cell culture**

The human squamous lung carcinoma cell line SW-1573 was grown in Leibowitz-15 medium (L15; Gibco-BRL life technologies, Breda, The Netherlands) supplemented with 10% fetal bovine serum and 2 mM glutamine at 37°C with no CO₂. The doubling time of the SW-1573 cells in exponential growth is 22–24 h.\textsuperscript{10}

For experiments, the cells were plated in 30 mm or 60 mm petri dishes (Costar Europe, Ltd., Badhoevedorp, The Netherlands) and grown to near confluence before irradiation to minimize possible reverse dose-rate effects and the effects of proliferation during irradiation. Because of this, the fraction of cells in S phase was reduced to 38 ± 4% compared to 58 ± 4% in exponentially growing cells. For sensitizing experiments, cells were incubated in medium containing 10 nM gemcitabine for 24 h before irradiation to ensure sufficient intracellular phosphorylation of gemcitabine.\textsuperscript{9} As a result, the cells became blocked in S phase; the fraction of cells in S phase after 24 h exposure was 77 ± 3%. Immediately before the start of irradiation, medium containing gemcitabine was removed, the cells were washed two times with PBS, and fresh medium was added.

**Irradiation**

Irradiations were performed with a Siemens Stabilipan ‘2’ X-ray machine (Siemens, Germany). Pulsed-LDR irradiation (p-LDR) were performed as described previously.\textsuperscript{26} Briefly, for p-LDR the pulse dose was 0.1 Gy at 9.16 cGy/min, and the resting period between the pulses was 4 min 52 s; this resulted in a mean dose rate of 1 Gy/h. The distance between focus and culture dish was 1.60 m, and a 1 mm Cu-filter was used. During irradiation, the cells were kept at 37°C in a water bath. Dose output was checked once a month with a BF-vat detector and a Farmer electrometer.

**Clonogenic assay**

Cell survival was determined by clonogenic assay. In brief, directly after irradiation the cells were trypsinized and replated in appropriate dilutions in six-well culture plates (Costar). Eight days later the colonies were fixed in 6% glutaraldehyde and stained with 0.05% crystal violet. Colonies of 50 cells or more were scored as originating from a single clonogenic cell. The plating efficiency of SW-1573 cells was 89 ± 12%. After the treatment of cells with 10 nM gemcitabine for 24 h, the plating efficiency was slightly reduced to 72 ± 13%.

Surviving fractions (S[D]/S[0]) after dose D, corrected for toxicity of gemcitabine alone, were calculated, and survival curves were analyzed by using Graphpad Prism statistical software (Graphpad Software Inc., San Diego CA, USA). The data were fitted to a pure exponential model, using only the linear term: S(D)/S(0) = exp – (αD)

**Metaphase slide preparation**

For metaphase slide preparation, SW1573 cells were plated in 100 mm culture dishes. Directly after p-LDR irradiation, the cells were transferred to 172 cm² tissue culture flasks. Twenty-four hours later, they were incubated for 2 hours with colcemid (0.1 µg/ml, Sigma), and the mitotic cells were shaken off. The mitotic cells were treated with hypotonic HCl for 10 min at 37°C and subsequently washed and fixed in methanol/acetic acid (3:1). The cells were dropped onto moist slides.

**Fluorescence in situ hybridization and scoring of color junctions**

Directly labeled whole chromosome-specific probes (chromosome 2-Cy3 and chromosome 18-FITC) were obtained from Cambio (Cambridge, UK). Metaphase double staining was performed with the method described by Pinkel et al.\textsuperscript{25} and Natarajan et al.\textsuperscript{26} An enhancement of the FITC signal was achieved with a commercially available amplification kit (Cambio). Metaphase slides were counterstained with DAPI (2.5 µg/ml) in PBS and embedded in an antifade solution (Vecta Shield, Vector Laboratories, Burlingame, CA, USA). The slides were examined by using a fluorescence microscope (Ortholux; Leica, Weltzlar, Germany) with a green light (552 nm) emission filter (615 nm) to detect Cy3 or an (495 nm) emission filter to detect FITC (519 nm). DAPI-stained metaphases were detected with a UV (372 nm) emission filter (456 nm). A total of 300 to 600 metaphases from three different experiments were scored for each dose and each chromosome. Aberrations were scored according to the PAINT method.\textsuperscript{27} The aberrations involving a painted chromosome and an unpainted chromosome were scored as color junctions, but the stable (one centromere) and the unstable aberrations (such as dicentrics) were listed separately. Stable-type aberra-
tions include translocations, deletions, and insertions. Dicentrics and acentric fragments are examples of unstable aberrations. In general, stable-type aberrations remain present in cells for several generations and correlate well with cell death, but unstable aberrations lead to cell death within 1 to 2 generations. Since scoring was performed in the first metaphase after irradiation, both stable and unstable aberrations could be scored. The incidence of unstable aberrations (such as dicentrics) in the number of color junctions in our experiment was very small (less than 4%). Fragments were easily distinguished from color junctions because they represent small pieces from a painted chromosome with only one color.

Three copies of chromosome 2 and two copies of chromosome 18 are present in SW-1573 cells. These chromosomes were chosen for analysis because in control cultures no aberrations were observed in these chromosomes. In total, SW-1573 cells contained 60 to 67 chromosomes.

The dose-effect curves for induction of color junctions by radiation were analyzed using Graphpad Prism statistical software. The data were fitted to a pure linear model: 

\[ F(D) = -\alpha D \]

A statistical analysis between the curves was performed with SPSS statistical software (SPSS 9.01 for Windows, Chicago, Ill., USA), using a stratified linear regression.

To determine the relative DNA content of chromosome 2 and chromosome 18, the length of all the chromosomes from 10 photographs of well-spread metaphases was measured, as described previously.

### RESULTS

#### Linear quadratic parameters and radioenhancement induced by gemcitabine

We calculated the linear quadratic parameters \( \alpha \) and \( \beta \) from previously published survival curves in the literature concerning radiosensitization by gemcitabine (see Table 1) according to the method described by Barendsen. In all cell lines the enhancement by gemcitabine is reflected by changes in the \( \alpha \)-parameter; moreover, the enhancement factor increases with increasing gemcitabine dose (Table 1).

No clear changes were apparent in the \( \beta \)-component.

### Table 2. Alpha parameters for cell survival and color junction induction after p-LDR irradiation with and without gemcitabine (dFdC). The enhancement factor (EF) calculated from these data (\( \alpha \) with/\( \alpha \) without dFdC) is also given. For the extrapolation of the \( \alpha \) values to that of the entire genome, see the text. Data \( \pm \) standard error.

<table>
<thead>
<tr>
<th>Pulsed LDR irradiation:</th>
<th>Control</th>
<th>dFdC</th>
<th>Enhancement factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell survival</td>
<td>0.29 ± 0.01</td>
<td>0.49 ± 0.01</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Cell survival (0–4 Gy)</td>
<td>0.21 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Chromosome 2</td>
<td>0.021 ± 0.001</td>
<td>0.027 ± 0.002</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Chromosome 18</td>
<td>0.014 ± 0.001</td>
<td>0.01 ± 0.001</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Total genome relative to 2</td>
<td>0.27 ± 0.01</td>
<td>0.35 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Total genome relative to 18</td>
<td>0.56 ± 0.04</td>
<td>0.4 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Radiation dose survival curves of SW1573 cells after p-LDR irradiation (a) with and without 10 nM gemcitabine for 24 h, and (b) enlargement of the initial part of the p-LDR irradiation survival curve. The data points represent the mean value from at least three experiments. The standard error is indicated by bars, but in several cases the s.e.m. is too small to be indicated in the figure.

#### Cell survival

The initial part of the radiation curves in the range of 0–4 Gy, both in the control and the gemcitabine-sensitized one, shows increased cell survival, and this leads to lower \( \alpha \)-values than those derived from the overall curve (Table 2). This confirms earlier results. After p-LDR irradiation, a clear radiosensitization by gemcitabine was observed (Fig. 1). The corresponding values of alpha and the enhancement factors are shown in Table 2. The enhancement factor as a result of irradiation (\( \alpha \) plus gemcitabine/\( \alpha \) minus gemcitabine) was 1.6 ± 0.1 (0–4 Gy range) or 1.7 ± 0.1 (entire curve). The dose-modifying factor at the 10% survival level was 1.4.

#### Chromosome aberrations

Figure 2 shows the induction of color junctions and acentric fragments in chromosomes 2 and 18 after irradiation; the corresponding \( \alpha \)-values calculated from these curves are given in Table 2. An overview of color junctions and acentric fragments observed in chromosome 18 after 4 Gy can be found in Table 3.

No increase in color junctions in chromosome 18 was
observed after gemcitabine treatment, only an insignificant ($p = 0.63$) tendency to decrease. In chromosome 2 an increase was observed compared to radiation alone, but this increase was also statistically not significant ($p = 0.13$). With theacentric fragments, the changes from control that were observed for fragments from both chromosomes were significant: for chromosome 2 and an increase was observed ($p < 0.05$), and for chromosome 18 the number of fragments decreased after gemcitabine treatment ($p < 0.05$).

Based on their physical length, chromosomes 2 and 18 represent $7.8 \pm 0.6\%$ and $2.5 \pm 0.07\%$ of the entire genome. Extrapolation of the values of LQ parameters for the entire genome resulted in $\alpha$ values of $0.27 \pm 0.01$ and $0.56 \pm 0.04$ (Table 2).

**DISCUSSION**

We show that treatment with gemcitabine resulted in a clear enhancement of effects of p-LDR on cell survival. This was obvious from both the alpha obtained from the low dose part and the complete curve (Table 2). A small increased level of survival in the initial part of the p-LDR survival curve has been described previously.$^{29}$ The present data confirm the expectations based on our results on the enhancement observed at HDR irradiation.$^{29}$ However, the enhancement factor observed after 10 nM gemcitabine for 24 h, immediately followed by HDR irradiation, was 2.9, but after p-LDR the enhancement factor is only 1.7. It should be noted, however, that already the $\alpha$-values for control cells differ markedly between p-LDR and HDR, 0.29 and 0.10, respectively. The underlying mechanism is not clear. Possibly differences in the DNA damage induction, induced resistance, and resulting repair processes between the HDR and LDR treatment play a role. The enhancement factor observed by other authors in the literature for combined radiation and gemcitabine treatment varies from 1.4 to 18 (Table 1). McGinn et al.$^{30}$ even observed the very high value of 42 for the enhancement factor in a pleiotropic drug-resistant breast cancer cell line. In all cell lines reported, the enhancement factor increases with increasing gemcitabine dose (Table 1).

The data in Table 2 show a significant difference in the extrapolated $\alpha$ value (to the total genome) between chromosomes 2 and 18. Differences in radiosensitivity between different chromosomes have been explained by non random radiation-induced DNA damage and repair.$^{31}$ Previously we reported differences in sensitivity to HDR irradiation between chromosomes 2 and X.$^{32}$

In the present study we observed no consistent increase in
color junctions concomitant with radiosensitization. In chromosome 2 a small increase and in chromosome 18 a decrease in the number of color junctions were observed after radiation combined with gemcitabine compared to irradiation alone. These differences were not statistically significant. However, for the (unstable) acentric chromosome fragments from both chromosomes, significant changes were observed: in chromosome 2 an increase and in the case of chromosome 18 a decrease.

In our previous study using HDR irradiation, a small but significant decrease in color junctions in both chromosomes was observed with gemcitabine. We could not offer a clear explanation for this, but we suggested that the most severely damaged cells might be eliminated by apoptosis. We observed that treatment with gemcitabine alone and combined with radiation led to some increase in apoptosis, but not to an extent that it could offer an explanation for the decreased level of color junctions. So if apoptosis plays a role, it might explain only a small part of the decrease in color junctions after combined treatment. Changes in cell cycle distribution were unlikely to be also involved because the cell cycle distribution had almost recovered from the gemcitabine treatment at the time of sampling for color junction measurement.

In Table 3, the data of Rosier et al. on the aberrations of chromosome 8 in the SQD9 cell line and chromosome 6 in the SCC61 cell line and our results on chromosome 18 from the SW-1573 cell line for both HDR and p-LDR irradiation are summarized. The results of Rosier et al. show a clear increase in “overall” chromosome aberrations after combined gemcitabine radiation treatment, and on further analysis they find that this increase is the result of an increase in the number of acentric fragments. The authors conclude from these results that radioenhancement by gemcitabine is associated with a high frequency of residual chromosomal aberrations, suggesting an effect of gemcitabine on the repair of genomic lesions inducing secondary chromosome breaks. This is in evident contrast to our present findings at LDR and our previous findings at HDR. In another cell line derived from a human glioblastoma, we do not even see radioenhancement in confluent cells with the fraction of cells in S-phase reduced to 13 ± 4%. Although Rosier et al. use confluent cells, irradiation takes place with trypsinized cells in suspension. This difference in methodology may partly explain the differences in results; more important is still the difference in gemcitabine concentration and the time of incubation before irradiation. We used 10 nM gemcitabine applied for 24 h before irradiation, whereas Rosier et al. used 5 µM for 3 h. It may well be that after such a high concentration of this drug, other mechanisms of action are involved.

Rosier et al. also investigated DNA damage repair, using pulsed-field gel electrophoresis, and in the two cell lines studied they found that gemcitabine did not lead to changes in radiation-induced DNA double-strand breaks (dsb’s). These results contradict with recent findings of Weiss et al., which show a significant increase in radiation-induced residual DNA damage, assayed with constant field gel electrophoresis, after the addition of gemcitabine. Weiss et al. used 0.5 µM to even 1 mM gemcitabine applied for only 2 h to the cells before irradiation and observed a concentration-dependent increase in the number of DNA dsb’s. They concluded that DNA repair inhibition is the major mechanism of gemcitabine radiosensitization.

To summarize, although it is clear that gemcitabine-induced radiosensitization can be expected when combined with brachytherapy, as with HDR, the mechanism of radiosensitization is not evident, further experiments will be needed to elucidate this.

<table>
<thead>
<tr>
<th></th>
<th>SW-1573 Chromosome 8 (4 Gy, HDR)</th>
<th>SW-1573 Chromosome 18 (4 Gy, p-LDR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dFdC (5 µM) RT (5 Gy) dFdC+RT</td>
<td>dFdC (10 nM) RT (4 Gy) dFdC+RT</td>
</tr>
<tr>
<td>No. of metaphases</td>
<td>100 (n = 4) 201 (n = 4) 182 (n = 4)</td>
<td>400 (n = 2) 392 (n = 3) 400 (n = 2)</td>
</tr>
<tr>
<td>No. of aberrations/metaphase</td>
<td>0.05 0.52 0.72</td>
<td>0.01 0.18 0.12</td>
</tr>
<tr>
<td>Translocations</td>
<td>0.02 ± 0.01 0.2 ± 0.03 0.23 ± 0.01</td>
<td>0.005 ± 0 ± 0.13 ± 0.01 ± 0.09 ± 0.004</td>
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<tr>
<td>Dicentrics</td>
<td>0 ± 0 0.13 ± 0.01 0.13 ± 0.02</td>
<td>0 ± 0 ± 0.04 ± 0.005 ± 0.06 ± 0.004</td>
</tr>
<tr>
<td>Acentric fragments</td>
<td>0.03 ± 0.01 0.19 ± 0.01 0.37 ± 0.04</td>
<td>0.01 ± 0.01 ± 0.08 ± 0.01 0.19 ± 0.01</td>
</tr>
</tbody>
</table>

Table 3. Summary of the chromosome aberrations induced by exposure to gemcitabine (dFdC) to X-irradiation or both combined as observed by Rosier et al. in two cell lines compared with our previous results at HDR and the present results at p-LDR. Standard errors are from the original data. Apart from the total number of metaphases, the number of independent experiments (n) from which these originate is also indicated.
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