MAŁGORZATA WALICKA, EWA GODLEWSKA and HANNA KLECZKOWSKA

EFFECT OF UVC AND araC ON L5178Y-R AND L5178Y-S CELLS. NUCLEOID SEDIMENTATION

Department of Radiobiology and Health Protection, Institute of Nuclear Chemistry and Technology, ul. Dorodna 16; 03-195 Warszawa, Poland

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The effects of UVC radiation ($\lambda = 254$ nm, 85 J/m$^2$) and/or 1-$\beta$-D-arabinofuranosylcytosine (araC, $2 \times 10^{-3}$ M, 2 h) on two mouse lymphoma cell lines, UVC-sensitive and X-ray resistant L5178Y-R and UVC-resistant and X-ray sensitive L5178Y-S, were investigated. AraC treatment inhibited the semiconservative DNA replication to 1.4% and 3.8% in L5178Y-R and L5178Y-S cells, respectively, and decreased the sedimentation distance of nucleoids from the cells of both lines. The shortening of sedimentation distances induced by UVC and araC treatment was 8.1 mm for L5178Y-R cells and 11.8 mm for L5178Y-S, and indicated a higher number of DNA breaks in L5178Y-S cells. Assuming that such breaks are the result of the inhibition of DNA repair replication by araC, we conclude that L5178Y-S cells have a greater number of repaired sites than L5178Y-R cells.

The two sublines of mouse lymphoma cells L5178Y-R and L5178Y-S differ in sensitivity to UVC$^1$ radiation (mean lethal doses: 3.6 and 8.5 J/m$^2$, respectively [1, 2] and to several other DNA damaging agents [2, 3]. Difference in the cytotoxic effect of UVC on L5178Y-R and L5178Y-S cells may be related to the differences in the DNA repair capability. It was found that UVC-resistant L5178Y-S cells replicate UVC-damaged DNA in shorter fragments than the undamaged DNA (control). On the other hand UVC-irradiation has no effect on the size of DNA fragments

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$^1$ Abbreviations used: UVC, ultraviolet radiation, $\lambda = 254$ nm; araC, 1-$\beta$-D-arabinofuranosylcytosine; dThd, thymidine; Tris, 2-amino-2 (hydroxymethyl) 1,3-propanediol; EDTA-Na$_2$, ethylenediaminetetraacetic acid disodium salt; SDS, sodium dodecyl sulfate; c.p.m., counts per minute; r.p.m., revolutions per minute; PPO, 2,5-diphenyloxazole; POPOP, 1,4-di-2-(5-phenyloxazoly)benzene; EB, ethidium bromide; bp, base pair.
synthesized by L5178Y-R cells [3]. This observation was considered to be indicative of the different character of replication on damaged DNA templates in these cell sublines.

L5178Y-S cells are less mutable by UVC than L5178Y-R cells [4]. This suggests that the level of excision repair, which has been proved to be error-free [5] is higher in L5178Y-S than in L5178Y-R cells. Sensitive methods have to be used to examine this possibility, because the level of the pyrimidine dimer excision in L5178Y cells is low [6]. One such method commonly applied in the DNA repair studies is nucleoid sedimentation [7, 8].

The term “nucleoids” was introduced by Cook et al. [9] and refers to structures formed from eucaryotic cells under mild lysis conditions: in non-ionic detergents and 1 - 2 M NaCl. In 2 M NaCl they are depleted of histones and other chromatin proteins, but contain the nuclear matrix, DNA and almost total nuclear RNA [9, 10]. These structures sediment similarly to supercoiled circular DNA molecules.

DNA in nucleoids assumed to be arranged in loops connected with nuclear matrix. The number of loops in the cell nucleus is known for human cells and is estimated at approximately 50 000 [11, 12, 13], with the length of 25 000 - 50 000 bp. The loss of the superhelical structure, i.e. relaxation, may be induced by a single strand break in the double helix. Sedimentation studies indicate that relaxation occurs in the so called “supercoiled domain” of the molecular mass of $10^8$ - $10^9$ Da [14 - 19], corresponding to at least ten loops. Nucleoids with relaxed supercoiled domains sediment more slowly than the intact ones. Cook & Brazell [7] used this feature to examine DNA repair in mammalian cell. The method of Cook & Brazell is very sensitive, detects even about 200 single strand breaks per cell nucleus, but does not allow for direct quantitative determination of DNA breaks. Qualitatively, it gives results similar to the alkaline gradient sedimentation [20] and DNA strand separation (or DNA unwinding technique) [21]. This last method allows to quantify as small number of DNA breaks as that mentioned above. Using the nucleoid sedimentation or DNA unwinding technique to examine post-UVC excision repair one can register DNA strand breaks introduce by the endonuclease in the vicinity of the photodamaged bases. However, these breaks are transient due to subsequent repair replication and rejoining of newly polymerized patch to the parental DNA. Therefore, to make such breaks detectable, repair replication should be inhibited. In the case of UVC-induced damage inhibitors of polymerase α, mainly araC, or aphidicolin are applied.

Using the DNA unwinding technique to UVC irradiated and araC treated L5178Y cells Szumiel et al. (in preparation) found a higher number
of DNA breaks, i.e. a higher number of repaired sites, in L5178Y-S cells as compared with L5178Y-R cells. Results of parallel experiments, carried out with the nucleoid sedimentation technique, are reported in this paper.

MATERIALS AND METHODS

Cell culture. UVC-irradiation and araC treatment. L5178Y cells were cultivated as suspension in Fischer’s medium with 8% of bovine serum [23]. Five milliliter portions of $2 \times 10^5$ cells/ml in the medium were irradiated on Petri dishes, using TUV30 lamp (Philips, Eindhoven, The Netherlands). Irradiation in the medium was necessary due to the fragility of L5178Y cells in salt solutions [23]. Fluence rate was 1.06 J/m² as measured by uridine actinometer [24 and Z. Zarębska - personal communication]. Because of the strong absorption of UVC in the Fischer’s medium (2 mm layer absorbs 92% of this radiation [25]) samples were irradiated with 85 J/m². Survival of L5178Y cells irradiated under these conditions was estimated as $10^{-5}$ for L5178Y-R cells and $5 \times 10^{-2}$ for L5178Y-S cells. After irradiation cell suspensions were diluted with two volumes of the medium alone, or the medium containing $4 \times 10^{-3}$ M araC (Sigma, St. Louis MO, U.S.A.) and incubated at 37°C.

DNA synthesis. Portions of cell cultures, 5 ml, $2 \times 10^5$ cells/ml, were exposed to 85 J/m² UVC. Irradiated cell suspensions were diluted with two volumes of the medium alone or the medium containing $4 \times 10^{-3}$ M araC, and incubated for 2 h in the presence of 2.5 μCi/ml of $[^3H]$dThd [22 Ci/mM, UVVVR, Praha, Czechoslovakia]. Incubation was stopped by adding 16 ml of cold 8°C physiological saline. Samples were centrifuged and 5 ml of cold 5% trichloroacetic acid was pipetted to the cell pellets. Samples were left for 18 h at 4°C, then centrifuged, washed twice with methanol, dried, dissolved in 0.4 ml of 0.3 M NaOH and left at 37°C overnight. Two 0.1 ml portions of this solution were taken for radioactivity measurements in liquid scintillator (3.8 g PPO, 0.2 g POPOP per 11 of toluene mixed with Triton X-100 1:1), in Beckman counter type LS 8100. From the rest of the sample two 0.1 ml portions were taken for protein determination by Lowry’s method [26]; protein concentration was not determined using the standard curve, only the absorbance of the sample at 625 nm was recorded. As a measure of the DNA synthesis in cell cultures, UVC and/or araC treated or control, the ratio of the $^3$H-radioactivity of the sample to its absorbance at 625 nm was taken.

Nucleoids. For nucleoid sedimentation experiments cells were prelabelled for 20 - 21 h with 0.5 μCi/ml of $[^3H]$dThd [22 Ci/mM, UVVVR, Praha, Czechoslovakia] and, if needed, additionally with 0.5 μCi/ml of d-[^14C]leucine
[uniformly labelled, 348 mCi/mM, Amersham, England]. After labelling, cell suspensions were centrifuged and cells resuspended in a fresh portion of the medium. Cell suspensions were UVC irradiated and/or araC treated as described above. After 2 h incubation at 37°C 10 ml portions of cold 8°C physiological saline were added to 5 ml portions of incubated cell suspensions. Suspensions were centrifuged, supernatants discarded, and the cell pellets were left in about 0.3 ml of the medium. Then 50 μl of the cell suspension containing 1×10⁵ - 4×10⁵ cells was pipetted on 150 μl of the lysing layer: 1.5 M NaCl, 0.02 M EDTA-Na₂, 2.66 mM Tris, pH 8, 0.7% Triton X-100, on the top of a linear sucrose gradient. Gradients, total volume 4.6 ml, contained 1.5 M NaCl, 0.01 M Tris, pH 8, 0.001 M EDTA-Na₂, 15 - 30% of sucrose. Lysis was carried out at 4°C for 30 min. Gradients were run in SW55Ti rotor in Beckman L8-55 centrifuge for 1 h at 7000 r.p.m. at 4°C. Gradients were fractionated beginning from the bottom of the tube, on glass fiber filters (∅ = 23 mm) made of GF/C Whatman filter paper. The radioactivity on filters was measured after drying, in 10 ml of toluene scintillator (2.5 g PPO per 11 of toluene) in Beckman LS 8100 counter.

RESULTS

Effect of UVC and araC on DNA synthesis

Incorporation of [³H]dThd into the cell cultures after irradiation and/or araC treatment, relative to the untreated control population, is given in Table 1. The UVC-induced inhibition of DNA synthesis was similar in both cell lines and amounted to about 30%. Under the conditions used, araC efficiently inhibited [³H]dThd incorporation into L5178Y cells. The residual level of incorporation was higher in L5178Y-S (3.8%) than in

Table 1

<table>
<thead>
<tr>
<th>Relative (% of control) incorporation of [³H]dThd (ratio of radioactivity of the sample to its absorbance at 625 nm) into the L5178Y cells treated with UVC and/or araC</th>
</tr>
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<tbody>
<tr>
<td>Cell line</td>
</tr>
<tr>
<td>L5178Y-R</td>
</tr>
<tr>
<td>L5178Y-S</td>
</tr>
</tbody>
</table>

* UVC, population irradiated with UVC; araC, population treated with araC; UVC+araC, UVC irradiated and araC treated population.
L5178Y-R cells (1.4\%). The difference between L5178Y-R and L5178Y-S cells is statistically significant; Student's t-test, 0.01 < p < 0.02. Combined treatment with UVC and araC raised the residual level of thymidine incorporation in L5178Y-S cells to about 10.3\% and did not affect the residual incorporation in L5178Y-R cells (1.4\% and 1.2\%).

**Nucleoid sedimentation**

*Characteristics of nucleoids.* Examples of sedimentation profiles of the lysis products from the cells of both L5178Y lines are shown in Fig. 1. In the case of cells labelled with \[^{3}H\]dTd and \[^{14}C\]leucine Fig. 1A.

![Graph of sedimentation profile](image)

Fig. 1. An example of sedimentation profiles of the lysis products from L5178Y cells. A. L5178Y-R cells were labelled with 0.5 \(\mu\)Ci/ml of \[^{14}C\]leucine and 0.5 \(\mu\)Ci/ml of \[^{3}H\]dTd for 20 - 21 h. B. L5178Y-R cells labelled with 0.5 \(\mu\)Ci/ml of \[^{3}H\]dTd for 20 - 21 h.

The level of \(^{14}C\)-radioactivity in the main peak (see Table 2) is low. This indicates that the main peak contains mostly DNA; \(^{14}C\)-radioactivity at the top of the gradient derives from the degraded proteins and \(^{3}H\) - c.p.m., from the overlapping of \(^{14}C\) - c.p.m. in \(^{3}H\) channel of the scintillation counter. \(^{14}C\) content in the arbitrarily selected main peak (Table 2) was 2.18 ± 0.78\% of total \(^{14}C\)-radioactivity in the gradient for L5178Y-R and 3.10 ± 0.91\% for L5178Y-S cells.

These values are low in comparison with the values of 5 - 10\% obtained by other authors. Cook & Brazell in their study on human cells [14] found 7 - 7.6\% of \[^{14}C\]leucine and 72 - 75\% of \[^{3}H\]dTd radioactivity in the nucleoid fraction i.e. in the main peak. We found 81\% of \[^{3}H\]dTd radioactivity in the main peak for both L5178Y mouse cell lines.
Table 2

Radioactivity (percentage of the total) in the main peak of the sedimentation profiles
95% confidence limits are given. Number of profiles analysed in brackets

<table>
<thead>
<tr>
<th>Line</th>
<th>$^{14}$C</th>
<th>$^{3}$H</th>
</tr>
</thead>
<tbody>
<tr>
<td>L5178Y-R</td>
<td>2.18±0.78</td>
<td>80.75±13.62</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>L5178Y-S</td>
<td>3.10±0.91</td>
<td>81.60±13.56</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

Addition of EB to the gradient (Fig. 2) induced changes in sedimentation which are characteristic of the circular and supercoiled DNA. The relative sedimentation, $d/d_c$, i.e. the ratio of the sedimentation distance in the presence of EB to the sedimentation distance in control gradient, depended on the EB concentration in a way similar to that observed for other mammalian cells. After the initial decrease, the $d/d_c$ value reached a minimum in the range of 1.5 - 5 µg EB/ml, and rose again at higher concentrations.

![Graph](image_url)

**Fig. 2.** The effect of ethidium bromide on nucleoid sedimentation. $d$, sedimentation distance in the presence of EB. $d_c$, sedimentation distance in the control gradient. Distance measured from meniscus (●), L5178Y-R cells (○), L5178Y-S cells

The above characteristics of structures formed from L5178Y cells during their lysis and sedimentation justifies referring to them as to nucleoids.

**Sedimentation of nucleoids from UVC and/or araC treated cell.** Examples of the sedimentation profiles obtained in experiments with UVC and araC are shown in Fig. 3. Two hour treatment with $2 \times 10^{-3}$ M araC caused a decrease in the sedimentation rate (Fig. 3A, C). Irradiation of cells with UVC (Fig. 3B, D) followed by 2 hour incubation induced a shortening of the sedimentation distance which was greater than in the case of araC treatment. The combined UVC and araC treatment caused the greatest shortening of the sedimentation distance.
Changes in sedimentation distances of nucleoids obtained from both cell lines are presented in Table 3. The effect of araC on control cells was greater for L5178Y-S than for L5178Y-R cells: \( d_{c} - d_{\text{araC}} = 10.5 \) mm versus 8.3 mm. The influence of UVC on nucleoid sedimentation was the same in L5178Y-R and L5178Y-S cells: the shortening of the sedimentation distance was about 13 mm, but the addition of araC to the medium for the period of postirradiation incubation induced greater shortening of the sedimentation distance for L5178Y-S than for L5178Y-R cells: \( d_{\text{araC}} - d_{\text{UVC+araC}} \) equalled 11.8 mm and 8.1 mm, respectively. The difference is statistically significant for \( p < 0.001 \) in the Student's t-test.

![Figure 3](image)

**Fig. 3.** Effect of araC and/or UVC on the sedimentation of nucleoids from L5178Y-R cells (A, B) and L5178Y-S cells (C, D). Cells were labelled with 0.5 \( \mu \text{Ci/ml} \) of \([^{3}H]\)-dThd for 20 - 21 h, then irradiated with UVC (85 \( \text{J/m}^2 \)) and/or araC treated \( (2 \times 10^{-3} \text{ M, 2 h}) \)

**DISCUSSION**

The main aim of the present study was to examine the possibility that L5178Y-R and L5178Y-S cells differ in the capability of excision repair of UVC-induced DNA damage. In order to do this, we inhibited DNA replication by araC treatment. We tested the effect of araC on semiconservative DNA synthesis in unirradiated cells before starting experiments with irradiated cells. Two hours treatment with \( 2 \times 10^{-3} \text{ M} \) concentration of araC caused a decrease in the \([^{3}H]\)-dThd incorporation to 1.2% in L5178Y-R and 3.8% in L5178Y-S cells (Table 1). These data
are compatible with those of Rogers et al. [27] who used L5178Y cells of different origin from ours. In spite of their close relation, the two L5178Y sublines may differ in the transport of araC into cells, in the level of araC phosphorylation and in the deoxycytidine nucleotide metabolism. These differences were not examined.

Combined treatment of L5178Y-S cells with UVC and araC raised the residual level of \(^{3}H\)dTd incorporation from 3.8% to 10.3%. A similar observation with araC has been interpreted as indicative of repair replication [28]. This is possible owing to the fact that repair replication in mammalian cells is inhibited by araC less strongly than the semiconservative replication. The residual level of repair replication after two hours of 10\(^{-3}\) M araC treatment of human cells is several times higher than that of semiconservative replication and amounts to about 30% [28]. Thus, it can be presumed that the increased \(^{3}H\)dTd incorporation in UVC-irradiated L5178Y-S cells is due to repair replication. The increase was not observed in L5178Y-R cells.

It is to be noted that changes in \(^{3}H\)dTd incorporation may also depend on the cell cycle and protein synthesis disturbances, as yet unknown, arising in the experimental conditions applied.

We assumed that repair replication in L5178Y cells, if present, would be inhibited by araC to a high degree and it would be possible to detect the accumulation of DNA breaks by nucleoid sedimentation method.

Using this method we found that the effect of araC on the structure of nucleoids of unirradiated cells as demonstrated by the changes in sedimentation distance was greater in L5178Y-S than in L5178Y-R cells (Table 3). We may explain this effect by a higher DNA synthesis rate in L5178Y-S cells: S-phase duration is 8.1 - 9.2 h in L5178Y-R and 6.8 h

**Table 3**

*Decrease in the sedimentation distance of nucleoids of L5178Y cells after araC and UVC treatment*<sup>a</sup>

*Standard deviation of the mean is given. The number of determinations in brackets.*

<table>
<thead>
<tr>
<th>Line</th>
<th>(d_{C-d_{araC}}) mm</th>
<th>(d_{C-d_{UVC}}) mm</th>
<th>(d_{araC-d_{UVC+araC}}) mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>L5178Y-R</td>
<td>8.3 ± 0.9 (10)</td>
<td>13.0 ± 1.6 (7)</td>
<td>8.1 ± 0.7 (6)</td>
</tr>
<tr>
<td>L5178Y-S</td>
<td>10.5 ± 1.2 (11)</td>
<td>12.5 ± 2.0 (9)</td>
<td>11.8 ± 0.9 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> C, control population; araC, population treated with araC; UVC, population irradiated with UVC; UVC+araC, UVC-irradiated and araC treated population;
in L5178Y-S cells, and the amount of DNA is about $8.9 \times 10^{-12}$ g/per cell for both lines. Therefore, more replication forks are at work in L5178Y-S cells and are disrupted by the inhibitor, causing the observed alteration in nucleoid sedimentation. Hence, in the case of proliferating cells treated with a DNA synthesis inhibitor, sedimentation of nucleoids is influenced by disturbances in the semiconservative replication. This aspect of nucleoid sedimentation has not been considered in the literature [29 - 32].

UVC-irradiation caused the same decrease in the sedimentation distance for both cell lines; thus, it seems that UVC-induced changes in chromatin conformation in L5178Y-R and L5178Y-S cells are very similar. These changes are the consequence of the DNA synthesis inhibition which was the same in both cell lines (Table 1), and of DNA repair. UVC causes permanent or transient block in the replication fork displacement; hence, as proposed by Painter [33], conditions for double strand break formation at junctions between replicon clusters are created. On the other hand, the UVC-induced DNA repair involves a topoisomerase II-like activity which relaxes DNA at the early stage of repair [34], and an endonuclease, which produces nicks in the vicinity of damaged sites. The above mentioned kinds of breaks are involved in loosening of the nucleoid structure and decreasing the sedimentation rate. In excision-proficient cells nicks are transient because of their subsequent patching. When nicks produced by endonuclease are accumulated due to the inhibition of repair replication by araC, nucleoids of L5178Y-S cells sediment more slowly (Table 3) than those from L5178Y-R cells, indicating the presence of a higher number of nicks in the former cells. Thus, we conclude that in L5178Y-R cells there is a lower level of the endonuclease activity.

This result, obtained at the “quasi-chromatin” level is consistent with the observation of Szumiel et al. (in preparation) who used the DNA unwinding technique. In those experiments L5178Y-R and L5178Y-S cells were irradiated under the same conditions with 85 J/m² and incubated for 0 - 3 h in the presence of $2 \times 10^{-4}$ M araC. In such araC-treated, UVC-irradiated cells after 2 h incubation 6 breaks per $10^{10}$ Da were found for L5178Y-R cells and 17 breaks per $10^{10}$ Da for L5178Y-S cells. This corresponded to about 270 and about 765 breaks per genome.

Taken together, results of Szumiel et al. and those presented in this paper indicate that the difference in UVC-sensitivity of L5178Y-R and L5178Y-S cells is accompanied by a different excision repair ability: there are more DNA strand incisions and therefore, a higher capacity for repair replication in the UVC-resistant L5178Y-S subline than in the UVC-sensitive L5178Y-R subline.

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REFERENCES


