Effect of Hypothermia and Hyperthermia on the Induction of Chromosome Aberrations by Adriamycin in Human Leukocytes

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ABSTRACT

When human leukocytes are treated with Adriamycin (ADR) for brief durations of 1 to 2 hr at concentrations ranging between 0.04 and 0.25 μg/ml, a dramatic reduction in the frequency of chromosome aberrations is observed in the cells treated at 4°C in comparison to those treated at 37°C. Conversely, a severalfold increase in the frequency of aberrations is found if temperature at the time of ADR treatment is raised to 43°C. At higher temperatures, the most dramatic increase is in the frequency of exchanges. These results point to a parallelism between these studies and those carried out previously for determining cell death with hyperthermia and ADR treatment. This effect on chromosome aberrations appears only if temperatures of 4 or 43°C are applied during the period of exposure of cells to ADR. No effect is evident if cells are posttreated at 4 or 43°C after ADR is removed from the medium. The question of true synergism versus “facilitation” of influx of ADR into cells is discussed in light of the information obtained by other workers on total quantities of the drug present in the cell.

INTRODUCTION

Hahn et al. (4) and Hahn and Strand (5) have demonstrated striking synergism between ADR2 and hyperthermia in relation to cytoxicity to EMT-6 mammary sarcoma cells and EMT-6 mammary sarcoma of mice. In one experiment HAI cells treated with 0.02 μg of ADR per ml at 43°C showed survival of only 4 × 10^-2 in contrast to 90% for heat control and 60% for ADR control at 37°C. Although the mechanism of such synergistic effect is not yet clear, fluorescence measurements showed an increased concentration of the anthracyclines in cells treated at 43°C, presumably because of increased permeability of the cells' plasma membranes. However, this situation of increased permeability can be reversed if cells are exposed to high temperature for a period much longer than 30 min.

Although synergism between chemicals and hyperthermia has been previously demonstrated (6), the experiments mentioned above raise questions of practical importance for chemotherapy as well as for basic cell biology, specifically, in view of the fact that ADR is one of the most promising antitumor drugs available at present (3). Since the primary mode of action of anthracyclines appears to involve intercalation of the molecule into the double helix of DNA (10, 16) and the antibiotic causes extensive structural damage to chromosomes, even at doses as low as 0.05 μg/ml (13, 15), it would be desirable to know if the clastogenic effects of ADR are potentiated with increased treatment temperature. This would be particularly true in view of the data that hyperthermia facilitates the entry of ADR into the cell. Conversely, a lowering of temperature to, say, 4°C during treatment should show drastic reduction in the frequency of aberrations induced by ADR.

MATERIALS AND METHODS

All experiments were carried out using leukocytes from normal human subjects of both sexes. Whole blood was cultured in chromosome Medium 1A (Grand Island Biological Co., Grand Island, N. Y., Catalogue 176) in 15-ml glass tubes containing 5 ml of medium, or in 250-ml glass flasks using 50 ml of medium. All cultures were started at 37°C. A stock solution of ADR (Farmitalia, Milan, Italy) was prepared in distilled water. In all treatments ADR solution was added at room temperature and then cultures were immediately transferred to the treatment temperature for a desired length of time. Treatments at 4°C were carried out with cultures on the bottom shelf of the refrigerator; for 23°C the laboratory bench at room temperature was used, and for temperatures higher than 37°C a water bath was utilized. In all cases the tubes or flasks were in aluminum foil during the course of treatment to minimize the effect of light, if any. Treatment temperatures varied between ±0.5°C.

The bulk cultures of cells in flasks ensured uniform exposure of every cell to the chemical. However, for purposes of removing the ADR-containing medium, the bulk cultures were divided equally into a certain number of tubes before centrifugation (and washing where necessary). The cells were arrested in metaphase with Colcemid for the last 2 hr of culturing. Other details of experimentation, e.g., concentrations of chemical used, the recovery period, etc. are provided at appropriate places under “Results.” Slides were stained with 3% Giemsa for 5 min.

Chromosome aberrations were scored from at least 100 metaphases, when available. While scoring, the aberrations were classed as chromatid and isochromatid deletions, chromatid- and chromosome-type exchanges, mono- and dicentric triradials, sister unions, and chromatid- or chromosome-type rings. However, for the sake of economy of space, the tables represent all deletions under “Fragments” and all types of aberrations requiring rejoining under “Exchanges.” In Tables 2 and 3, chromosome-type exchanges and rings have also been presented as a fraction of total exchange population to give the reader an idea of relative frequency and to emphasize the fact that most populations scored in here were in the first cell cycle.
RESULTS

A series of control experiments was carried out at 4 and 43° (treatments lasting 2 hr) after 24, 44, and 60 hr of initiation of culture at 37°. Various recovery periods (namely, 48, 40, and 20 hr), were tried and cells analyzed for aberrations. Only chromatid deletions with a rare chromatid exchange were observed in these slides. The frequency ranged between 0 to 6 aberrations per 100 cells analyzed per treatment. There was no consistency of aberrations increasing or decreasing with temperature. In view of these observations, it was concluded that treatment of cells at 4 or 43° for 2 hr does not alter the frequency of aberrations in relation to the tubes raised constantly at 37°. The details of data are not given here.

The second series of studies was aimed at comparing the effect of ADR treatments at 37° versus 4°. One expects a lower frequency of aberrations in cells treated at 4° than at 37° due to a multitude of factors, e.g., reduced uptake of the drug, lowered enzyme activity, slowing of the cell cycle, etc. The first experiment (Table 1, Subject A) with cells treated during 22 to 23.50 hr after culture initiation at 0.04 μg of ADR per ml supported the idea. The frequency of aberrations after 48-hr recovery is only 0.02 per cell in the 4° treatment compared with 0.07 per cell in the 37° treated material. These values are very similar to those found in another experiment (Table 1, Subject B). In another similar experiment where the recovery period was reduced to 24 hr, no scorable metaphases were available, indicating the delay induced by the anthracycline. However, when concentration of ADR was raised to 0.08 μg/ml with 48 hr of recovery, 0.12 aberration per cell was found in the sample treated at 37° compared with 0.02 per cell at 4° treatment. Thus, in spite of a very low frequency of aberrations per cell, there is little doubt that ADR treatment at 37° produces more aberrations than at 4°.

The frequency of aberrations was increased by raising the concentrations of ADR. As seen in Table 1, Subject C, treatment of leukocytes with 0.125 μg/ml during 24 to 26 hr with recovery of 41 hr raises the aberrations to 1.01 per cell at 37°, and to 0.23 aberrations/cell in 4° treatment. Similarly, when cells were treated with 0.18 μg/ml between 24 to 26 hr at 37 or 4° and harvested 41 hr later, a total of 0.97 aberrations/cell in 37° treatment was found compared with only 0.18 aberrations/cell in 4° treatment. This difference is widened when cells are treated with 0.25 μg/ml with a recovery of 41 hr. However, when cells were treated with 0.25 μg of ADR per ml between 66 and 68 hr and allowed to recover for 22 hr, the frequency of aberrations was not different between the 2 temperature regimes. This may be due to lack of time for most cells with chromosome damage to come to metaphase. Accordingly, a recovery period of 26 hr clearly indicates an increase in aberration frequency at 37 versus 4° (0.87 versus 0.23). The details of all these data are produced in Table 1.

Beside the overall decrease in the frequency of aberrations at low temperatures, an observation of interest in Table 1 is the drastic reduction in the relative frequency of aberrations requiring rejoining of broken ends of chromosomes. Thus, whereas chromatid exchanges had shown a highly reduced frequency in the 4° population, aberrations like rings and triradials were totally absent. Thus, the frequency of exchange configurations is either based upon the frequency of total aberrations in the cell or that low

Table 1
Comparison of frequency of chromosome aberrations in leukocytes treated with various concentrations of ADR at 37 or 4° for up to 2 hr and then transferred to 37° until harvest

<table>
<thead>
<tr>
<th>Subject</th>
<th>Concentration (μg/ml)</th>
<th>Treatment time (hr)</th>
<th>Temperature</th>
<th>Recovery (hr)</th>
<th>No. of cells aberrant/analyzed</th>
<th>Type and frequency of aberrations/cell analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.00</td>
<td>Control</td>
<td>37°</td>
<td>48</td>
<td>3/100</td>
<td>Fragments: 0.03, Exchanges: 0.01, Total: 0.04</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>22-23.5</td>
<td>37°</td>
<td>48</td>
<td>2/100</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.00</td>
<td>Control</td>
<td>37°</td>
<td>48</td>
<td>0/100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>22-23.5</td>
<td>37°</td>
<td>48</td>
<td>2/100</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.125</td>
<td>Control</td>
<td>37°</td>
<td>41</td>
<td>1/100</td>
<td>Fragments: 0.76, Exchanges: 0.25, Total: 1.01</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>24-26</td>
<td>37°</td>
<td>41</td>
<td>6/100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>24-26</td>
<td>37°</td>
<td>41</td>
<td>5/100</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.00</td>
<td>Control</td>
<td>37°</td>
<td>41</td>
<td>4/100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>66-68</td>
<td>37°</td>
<td>41</td>
<td>1/100</td>
<td></td>
</tr>
</tbody>
</table>

*Includes all types of aberrations requiring rejoining, namely, chromatid exchanges, chromosome exchanges, triradials, and sister unions.
temperature does not permit the rejoining of broken ends (see “Discussion”).

Once it was established that effectiveness of ADR at 37° is far greater than that at 4°, a series of experiments was undertaken to compare the effect of the drug at 37° and higher temperatures. In the first study, 4 tubes were set up at 37° and 0.2 µg of ADR per ml was added on the 27th hr of culture. Two of these were returned to 37° while the other 2 were incubated for 1 hr at 43° in a water bath. After 1 hr all 4 tubes were centrifuged, the medium was changed and reincubated at 37° until harvest. The data on chromosome aberrations (Table 2, Subject A) studied after recovery of 44 or 62 hr, exhibited a far greater potential of ADR in causing aberrations in synergism with hyperthermia (43°) than at 37°. Thus, at 44-hr recovery, not only the number of aberrant cells at 43° is 3 times as much as that at 37°, but the frequency of aberrations is about 9-fold as much as at 37°. The largest relative increase is found in the frequency of those aberrations that require rejoining (29-fold for exchanges versus 7-fold for free fragments), especially chromatid exchanges. However, it is possible that raised temperature during ADR treatment hastens the cell cycle and, hence, maximum possible frequency of aberrations for 37° treated material is not exhibited at 44-hr recovery. The data that follow for the 62-hr recovery period show that the frequency of aberrations for 37° treated material is now about 3-fold higher than for the same treatment at an earlier fixation time, whereas the reverse is true for cells treated at 43°. In spite of this, the absolute frequency of aberrations in hyperthermic material is still about twice as high as in the other. The differences in aberration frequencies in the 2 sets of treatments are dramatic, the higher of the 2 values for aberrations in 43° treated material is about 3.5 times that of 1 in 37°. Similarly, this is true of the number of aberrant cells.

A second experiment was performed by treating the cells at 24 to 25 hr postculture initiation period. This time the cells were treated with 0.2 µg of ADR per ml at 4, 23, 37°, and 43°. A recovery of 45 hr was allowed. The data (Table 2, Subject B) confirm the earlier findings: a slightly more than 2-fold increase in the frequency of aberrations in 43° treated material was found compared with the one at 37°. Also, a large increase (about 4-fold) was observed in the cumulative frequency of all types of exchanges (compared with 1.5-fold increase for free fragments). The temperatures lower than 37° had fewer aberrations (about 30%).

Although the data presented above make a case of increased effectiveness of ADR under hyperthermic situations and vice versa (Table 1), one still needs to carry out an experiment to see if the shift in cell cycle caused by high temperature does not result in a superficial increase in aberration frequency. One also wonders if 43° is not a unique temperature in this regard. Therefore, in the next experiment the cultures were treated for only 45 min (between 29'/4 to 30 hr) at 37 and 47°, and cells were recovered after 32, 40, 48, and 64 hr. In these studies it was also possible to see that higher frequencies of cells in 37° material were obtainable at 40-hr recovery (0.87 aberrations/cell) compared with the highest recovery in 47° material at 48 hr (1.25 aberrations/cell). Thus, at least at this temperature, if anything, cells have suffered a setback in mitotic progression. The results of the 2 most effective protocols in the instance of 37° and 3 protocols for 47° are presented in Table 2, Subject C. Again, the highest relative increase is in the frequency of exchange-type aberrations.

Human leukocytes in tissue culture initiate their DNA synthesis at around 26 to 30 hr after culture initiation (1, 11). Thus, none of the above studied population was well

<table>
<thead>
<tr>
<th>Subject</th>
<th>Concentration (µg/ml)</th>
<th>Time (hr)</th>
<th>Temperature</th>
<th>Recovery (hr)</th>
<th>% of aberrant cells/cells analyzed</th>
<th>Types and frequencies of aberrations/cell</th>
<th>Chromosome type/total exchanges</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
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<tr>
<td>A</td>
<td>0.2</td>
<td>27-28</td>
<td>37°</td>
<td>44</td>
<td>22/100</td>
<td>0.17 0.11 0.28 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>27-28</td>
<td>43</td>
<td>44</td>
<td>60/100</td>
<td>1.18 1.45 2.63 0.03</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>27-28</td>
<td>37</td>
<td>62</td>
<td>29/100</td>
<td>0.37 0.39 0.76 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>27-28</td>
<td>43</td>
<td>62</td>
<td>45/100</td>
<td>0.63 0.65 1.48 0.03</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.2</td>
<td>24-25</td>
<td>37</td>
<td>45</td>
<td>35/100</td>
<td>0.57 0.27 0.84 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24-25</td>
<td>4</td>
<td>45</td>
<td>21/100</td>
<td>0.18 0.07 0.25 0.08</td>
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<tr>
<td></td>
<td></td>
<td>24-25</td>
<td>23</td>
<td>45</td>
<td>15/100</td>
<td>0.20 0.01 0.21 0.07</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>24-25</td>
<td>43</td>
<td>45</td>
<td>49/100</td>
<td>0.81 1.01 1.62 0.01</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.1</td>
<td>29.25-30</td>
<td>37</td>
<td>40</td>
<td>35/100</td>
<td>0.40 0.37 0.77 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.25-30</td>
<td>37</td>
<td>48</td>
<td>19/100</td>
<td>0.19 0.11 0.30 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.25-30</td>
<td>47</td>
<td>40</td>
<td>34/100</td>
<td>0.38 0.10 0.48 0.01</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>29.25-30</td>
<td>47</td>
<td>48</td>
<td>47/100</td>
<td>0.55 0.70 1.25 0.19</td>
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<td></td>
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<td>29.25-30</td>
<td>47</td>
<td>64</td>
<td>35/100</td>
<td>0.48 0.13 0.61 0.00</td>
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</tr>
<tr>
<td>D</td>
<td>0.1</td>
<td>47-48</td>
<td>37</td>
<td>64</td>
<td>6/100</td>
<td>0.08 0.01 0.09 0.15</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>47-48</td>
<td>37</td>
<td>69</td>
<td>7/100</td>
<td>0.03 0.01 0.04 0.00</td>
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<tr>
<td></td>
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<td>47-48</td>
<td>43</td>
<td>64</td>
<td>25/100</td>
<td>0.29 0.24 0.53 0.03</td>
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<tr>
<td></td>
<td></td>
<td>47-48</td>
<td>43</td>
<td>69</td>
<td>21/100</td>
<td>0.18 0.10 0.28 0.02</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>47-48</td>
<td>43</td>
<td>74</td>
<td>6/100</td>
<td>0.04 0.04 0.08 0.03</td>
<td></td>
</tr>
</tbody>
</table>

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* — , no chromosome-type exchange was observed.
into S phase. This was achieved by treating the cells during the 47th to 48th hr of culturing with 0.1 μg/ml at 37 and 43° and providing much longer recoveries, up to 74 hr.

The data (Table 2, Subject D) were interesting in one regard, that the treatments produced a minimal number of aberrations in the cells of this subject at 37°. An increase at 43° would be a critical test of synergism. The highest frequency in hyperthermia population turned out to be about 6 times as much as in the normal 37° treatment (0.53 versus 0.09 per cell), with a dramatic increase in exchanges. Part of the data from this experiment are reproduced in Table 2.

The foregoing experiment permits the following conclusions that (a) hyperthermia increases the effectiveness of ADR in producing more aberrant cells with higher frequency of aberrations per cell; (b) highest relative frequency is in the population of exchanges; and (c) in spite of some effect on cell progression of hyperthermia, equivalent recovery periods for 43 and 37° show sufficient differences in breakage frequencies to establish the superior effectiveness of hyperthermia in combination with ADR in inducing karyotypic alterations of structural nature.

The data in the foregoing experiments leave 1 major question unanswered. Is the effect of hyperthermia really synergistic with that of ADR; i.e., does the observed enhanced effectiveness of ADR express itself in terms of induced chromosome aberrations at the time and for the duration of treatment, or is the effect delayed, expressing itself sometime during the recovery period due to alterations in the activity of the enzymes, etc. that act during recovery upon the lesions induced by ADR at the time of treatment? It is entirely possible that the major effect of hyperthermia is really to increase the inhibition of restitution of ADR-induced damage to the chromatin material. Distinction between immediate effectiveness of ADR and inhibition of repair at the posttreatment period can be easily made if cells are treated at 37° and then postincubated for a few hr at various temperatures. In case "delayed" effectiveness due to repair process is involved, the end results should be similar to those observed in Tables 1 or 2 for 4 or 43° posttreatment.

A series of experiments was conducted by treating the cells with 0.25, 0.02, or 0.1 μg of ADR per ml at 37° at 47 to 49, 24 to 26, and 48 to 50 hr, respectively, postculture initiation. After removal of ADR the cells were incubated further for 2 or 4 hr at 4, 23, 37, and 43°. Recoveries varied between 41 and 72 hr after removal of ADR. The protocols and details of the nature and frequencies of aberrations are tabulated in Table 3. There is no evidence that 4° posttreatment reduces the frequency of aberrations. Also, the frequency of aberrations is not enhanced in the material posttreated at 43°. Minor fluctuations as observed in the table for comparable treatments in any given experiment are expected on the basis of effects of different temperatures on cell cycle, or simply represent variations between tubes. Thus, in Experiment A, a decrease in aberrant cells and a slight increase in frequency of aberrations is found in the material posttreated at 4° than that at 37°. The same is true for 43° posttreatment and the data for 23° fall closer to those for 37°. Thus, there is no consistent increase or decrease with increasing temperature, as also found in Experiment B. Similarly, no case can be made for increased relative frequency of exchanges as observed previously at 43°.

The data in the 3 experiments (Table 3, Subjects A to C) do not show any temperature-dependent aberration frequency, even when cells are treated when no DNA synthesis supposedly takes place (Table 3, Subject B), or when the

<table>
<thead>
<tr>
<th>Subject</th>
<th>Concentration (μg/ml)</th>
<th>Treatment time (hr)</th>
<th>Temperature</th>
<th>Colcemid</th>
<th>No. of cells abnormal/analyzed</th>
<th>Types and frequencies of aberrations/cell</th>
<th>Chromosome type/total exchanges</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.25</td>
<td>47-49</td>
<td>37°</td>
<td>90-92</td>
<td>67/100</td>
<td>Breaks: 1.81, Exchanges: 1.27, Total: 3.08</td>
<td>0.02</td>
</tr>
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<td></td>
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<td></td>
<td>4</td>
<td>90-92</td>
<td>49/100</td>
<td>Breaks: 2.15, Exchanges: 1.70, Total: 3.85</td>
<td>0.04</td>
</tr>
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<td>23</td>
<td>90-92</td>
<td>63/100</td>
<td>Breaks: 1.45, Exchanges: 1.28, Total: 2.73</td>
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<td>43</td>
<td>90-92</td>
<td>67/100</td>
<td>Breaks: 2.13, Exchanges: 1.44, Total: 3.57</td>
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<tr>
<td>B</td>
<td>0.20</td>
<td>24-26</td>
<td>37°</td>
<td>70-72</td>
<td>17/100</td>
<td>Breaks: 0.17, Exchanges: 0.07, Total: 0.24</td>
<td>0.28</td>
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<tr>
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<td>4</td>
<td>70-72</td>
<td>25/100</td>
<td>Breaks: 0.31, Exchanges: 0.12, Total: 0.43</td>
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<td>23</td>
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<td>Breaks: 0.44, Exchanges: 0.25, Total: 0.69</td>
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<td>43</td>
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</tr>
<tr>
<td>C</td>
<td>0.10</td>
<td>48-50</td>
<td>37°</td>
<td>94-96</td>
<td>40/100</td>
<td>Breaks: 0.54, Exchanges: 0.16, Total: 0.70</td>
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<td>43</td>
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<td>Breaks: 0.46, Exchanges: 0.13, Total: 0.59</td>
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<td>94-96</td>
<td>52/100</td>
<td>Breaks: 0.74, Exchanges: 0.18, Total: 0.92</td>
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<td></td>
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<td>43</td>
<td>94-96</td>
<td>42/100</td>
<td>Breaks: 0.61, Exchanges: 0.23, Total: 0.84</td>
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<td></td>
<td></td>
<td></td>
<td>37</td>
<td>122-124</td>
<td>40/100</td>
<td>Breaks: 0.48, Exchanges: 0.22, Total: 0.70</td>
<td>0.14</td>
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<td>4</td>
<td>122-124</td>
<td>58/100</td>
<td>Breaks: 1.07, Exchanges: 0.18, Total: 1.25</td>
<td>0.05</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>43</td>
<td>122-124</td>
<td>49/100</td>
<td>Breaks: 0.58, Exchanges: 0.18, Total: 0.76</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a Time refers here to postculture hr; oh, culture initiation time.
b —, no chromosome-type exchange was observed.

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cells constitute an asynchronous population after completing the first cycle (Table 3, Subject C). Also, there is no apparent consistent effect of varying duration of posttreatment (2 versus 4 hr) or recovery (44 versus 72 hr) as seen in Table 3, Subject C.

Thus, it may be concluded that the major effect of ADR is during the period when the chemical is in contact with the cells, or, alternately, that any changes in enzymatic activity, etc. brought about by temperature fluctuations in the post-ADR treatment period do not have any serious effect on the frequency of aberrations induced by this anthracycline.

Considering the delay caused in the cell cycle by ADR as discussed above, and the recovery of none or relatively very few chromosome-type exchanges compared with all cells, or, alternately, that any changes in enzymatic activity, etc. brought about by temperature fluctuations in the post-ADR treatment period do not have any serious effect on the frequency of aberrations induced by this anthracycline.

The experiments with hyperthermia are instructive as guidelines in chemotherapy. These are significant from the cell biology point of view since cell death could result from chromosome damage, resulting in inhibition of proliferation of the cell and eventual death during mitosis or soon after it. It is not known, however, what proportion of cells actually die from such a mechanism. Also, the role of chromosomal nondisjunction, as known to result from anthracycline treatment (8), has not been assessed as a cause of cell death. A correlation between these genetic parameters [along with problems associated with DNA repair, since anthracyclines can cause single- as well as double-strand breaks (2)] and cell death may answer some of these questions.

Data from Newsome and Littlefield (9) with skin fibroblasts differ from those of Vig (13) using leukocytes, in that in the former case very few exchanges were induced in ADR-treated materials. The high incidence of exchanges induced in the present experiments at 43°, but fewer exchanges at 4°, indicate that exchange frequency is dependent upon total frequency of aberrations and may well correlate with the increased uptake of ADR by the cell. It can also be true for reduced quantities of ADR in the cell at 4° treatment. The length of the recovery period, time of treatment, etc. also promote higher frequency of exchanges (see e.g., Ref. 15).

Thus, it may be concluded that the major effect of ADR is during the period when the chemical is in contact with the cells, or, alternately, that any changes in enzymatic activity, etc. brought about by temperature fluctuations in the post-ADR treatment period do not have any serious effect on the frequency of aberrations induced by this anthracycline.


Effect of Hypothermia and Hyperthermia on the Induction of Chromosome Aberrations by Adriamycin in Human Leukocytes

B. K. Vig


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