Intrinsic Differences in Heat and/or X-Ray Sensitivity of Seven Mammalian Cell Lines Cultured and Treated under Identical Conditions

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ABSTRACT

The variation in heat and/or X-ray response of 7 mammalian cell lines treated under identical culture conditions and experimental procedures was examined. Large differences in thermal response at 42.5 and 45.5°C (dose-modifying factors of greater than 10) were observed among the cell lines, and the order of heat sensitivity for the 7 cell lines was similar but not identical at 42.5 and 45.5°C. However, classification of thermal sensitivity at 42.5°C depended on the time of heating, since thermal tolerance developed at different survival levels after 3 to 4 hr of heating for pig kidney, muntjac, Chinese hamster (V79 and CHO), and HeLa cells, whereas no thermal tolerance and only a transitory thermal tolerance were observed for mouse LP59 and rat kangaroo cells, respectively. Also, Chinese hamster ovary cells were more heat sensitive when cultured in McCoy's Medium 5a containing 10% calf serum plus 5% fetal calf serum than when cultured in F12 medium containing 10% fetal calf serum.

Small variations in the X-ray dose response of the seven mammalian cell lines (dose-modifying factors of less than 1.5) were observed. The X-ray response was enhanced by thermal treatment at 42.5°C for 1 hr preceding irradiation, and there was a positive correlation between the degree of thermal enhancement and cellular thermal sensitivity at 42.5°C. However, there was no correlation between differences in heat sensitivity and differences in radiosensitivity. Also, no apparent correlation between heat and/or X-ray response and cell chromosome number, DNA content, cell volume, and cell population doubling time could be found. However, heat sensitivity could, in general, be related to the body temperature of the species from which the cell line was derived.

INTRODUCTION

Hyperthermia either by itself or as an adjunct to X-irradiation is being investigated as a modality for cancer therapy (6, 7, 25, 31). Studies with mammalian cells cultured in vitro (1, 7, 17, 20, 29, 34, 37) show that the many cell lines used, such as pig kidney, V79, CHO, EMT6, and HeLa, all appear to have different heat sensitivities (16, 17, 23, 24, 34). However, even the same cell line studied in different laboratories seems to have different heat responses, e.g., comparisons with HeLa cells (22, 23, 29), pig kidney cells (17, 33), V79 cells (16, 36), and CHO cells (7, 20). Some studies also have shown that malignant cells cultured in vitro are more sensitive to heat treatment than the normal cell lines (11, 21, 26, 27).

Because of the differences in cellular heat responses among different laboratories, it is difficult to ascertain whether the differential heat sensitivity in the malignant cells versus normal cells and of the different cell lines is due to an inherent cellular property or due to different culturing and medium conditions used in the various laboratories. The data presented by Freeman et al. (9), Gerweck (10), Hahn (12), Henle and Dethlefsen (19), Overgaard and Bichsel (28), Power and Harris (30), Raaphorst and Dewey (32), and Schlag and Lücke-Huhle (36), demonstrate that such factors as nutritional state, growth phase, pH, oxygen concentration, and tonicity can affect the heat sensitivity of cultured mammalian cells. Therefore, differences in these environmental factors in cell cultures may produce different thermal responses in the various cell lines, thus making it difficult to determine differences in the inherent heat sensitivity of the different cells.

A study by Bhuyan et al. (2) has shown that the heat responses of various cell lines cultured in the same laboratory are different. Although 2 of the cell lines, HeLa and L1210, were cultured in the same medium, the HeLa cells were cultured in Roswell Park Memorial Institute medium in monolayer while the L1210 cells were grown in suspension in Roswell Park Memorial Institute medium. Thus, the different media and culture conditions may be responsible for the observed differences in heat sensitivity. In another investigation by Harisiaiadis et al. (15), in which 4 cell lines were maintained in the same medium, except for the addition of Eagle's minimal essential medium and L-glutamine for 2 cell lines, a very similar heat response was observed for CHO, V79, RLB normal liver, and Morris rat hepatoma cells.

In this study, we investigated the inherent differences in heat and/or radiosensitivity of 7 mammalian cell lines (CHO-10, V79, HeLa S3, mouse LP59, pig kidney CCL33, muntjac CCL157, and rat kangaroo CCL56) cultured in exponential growth phase in attached monolayer using F12 medium. All culture conditions were carefully controlled so that all cell lines were cultured in an identical manner, and all experimental procedures were identical for all the cell lines.

MATERIALS AND METHODS

The pig kidney CCL33, muntjac CCL157, and the rat kangaroo CCL56 cell lines were purchased from the Ameri-
Heat and/or X-Ray Sensitivity in Seven Cell Lines

The data depicted in Chart 1 show that the hyperthermic response of CHO cells can be altered depending on the medium in which the cells were cultured. At heating times greater than 4 hr, the survival of cells cultured in F12 medium was 5 times higher than that of cells cultured in McCoy's Medium 5a. In both types of media, the CHO cells heated for more than 4 hr show a thermal-tolerant plateau which is routinely observed by others (7, 10, 29). Thermal tolerance occurred sooner when cells were treated in F12 medium. Variations in thermal response with the use of several different media have also been observed by others (D. P. Highfield and W. C. Dewey, personal communication).

Charts 2 and 3 show the survival of the 7 cell lines after hyperthermic treatment at 45.5 or 42.5°C, respectively. Large differences in heat sensitivity can be observed among the various cell lines. The order of heat sensitivity, from most to least sensitive, at 45.5°C is V79, CHO, mouse LP59, HeLa S3, rat kangaroo CCL56, muntjac CCL157, and pig kidney
G. P. Raaphorst et al.

LP59, CHO, HeLa S3, V79, muntjac CCL157, and pig kidney CCL33 cells. However, the rat kangaroo CCL56, compared to the V79, CHO, and HeLa S3 cell lines, appears to be more resistant for heating intervals between 2 and 5 hr but more sensitive for heating intervals longer than 6 hr. Thermal tolerance occurs for heating intervals longer than 4 hr for all the cell lines, except the mouse LP59 and the rat kangaroo CCL56 cells.

The radiation survival data for the 7 cell lines are shown in Chart 4. There are differences in the radiosensitivity of the various cell lines, with the HeLa cells showing the lowest survival and the V79 cells showing the highest survival. The survival curve parameters are listed in Table 2. The effect of heat treatment for 60 min at 42.5° on the cellular radiosensitivity is shown in Chart 5. It is of interest to observe by comparing Charts 4 and 5 that the order of radiosensitivity for the 7 cell lines has been altered by the heat treatment. Also, the initial heat exposure results in a greater range in radiosensitivities.

In Table 1, several characteristics of the 7 cell lines are listed and compared to the resistance to hyperthermic treatment. The data demonstrate that there is no apparent correlation between thermal sensitivity and cell volume, cell population doubling time, plating efficiency, chromosome number, or the average DNA content per cell in an asynchronous population. The G1 DNA content was also calculated using cell cycle parameters and the mathematical formulation presented by Elkind and Whitmore (8); no correlation between G1 DNA content and heat sensitivity was found. The DNA content was measured using the diphenylamine assay as described by Burton (4). However, there appears to be a correlation between heat sensitivity and body temperature of the donor species from which the cell line was derived. The pig has the highest body temperature, and pig kidney cells are most heat resistant. Also, cells from the muntjac, which has a relatively high body temperature, are quite resistant. The HeLa S3, mouse LP59, and CCL33 cells. The survival curves for all the cell lines except the HeLa S3 possess a shoulder. The lack of a shoulder in heat survival curves for HeLa cells also has been observed by others (21). Survival curve slopes have not been calculated because the data are not linear on a semilogarithmic plot. However, the continual downward curvature at low survival is probably not due to cell density effects because survival from heat and/or radiation treatments was slightly higher when 10⁴ to 10⁶ cells were inoculated per flask, whereas no cell density effect was observed when cell numbers were less than 10⁴ as determined in other experiments.

When cells were heated at 42.5° (Chart 3) large differences in heat sensitivity were also observed, and the order of heat sensitivity of the cell lines is similar but not identical to that for cells heated at 45.5° (Table 1). At 42.5°, the order of thermal sensitivity from most to least sensitive is mouse LP59, CHO, HeLa S3, V79, muntjac CCL157, and pig kidney CCL33 cells. However, the rat kangaroo CCL56, compared to the V79, CHO, and HeLa S3 cell lines, appears to be more resistant for heating intervals between 2 and 5 hr but more sensitive for heating intervals longer than 6 hr. Thermal tolerance occurs for heating intervals longer than 4 hr for all the cell lines, except the mouse LP59 and the rat kangaroo CCL56 cells.

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Comparison of heat sensitivity and cell line parameters

<table>
<thead>
<tr>
<th>Order of thermal resistance&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relative cell volume&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Population doubling time (hr)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Av. plating efficiency (%)</th>
<th>Diploid no. of donor mammal&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Range of 90% of the cells&lt;sup&gt;df&lt;/sup&gt;</th>
<th>Maximum fraction&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Av. DNA content of an asynchronous cell population (g/cell x 10&lt;sup&gt;-11&lt;/sup&gt;)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Body temperature of donor mammal&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig kidney CCL33</td>
<td>42.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.46</td>
<td>26</td>
<td>42.7</td>
<td>40 35-55</td>
<td>49% at 36-38</td>
<td>2.07</td>
<td>39.4</td>
</tr>
<tr>
<td>Muntjac CCL157</td>
<td></td>
<td>1.43</td>
<td>28</td>
<td>52</td>
<td>7 5-8</td>
<td>75% at 7</td>
<td>1.21</td>
<td>38.5</td>
</tr>
<tr>
<td>HeLa S3</td>
<td></td>
<td>1.62</td>
<td>22</td>
<td>65.8</td>
<td>46 47-75</td>
<td>Extremely aneuploid</td>
<td>2.36</td>
<td>37.0</td>
</tr>
<tr>
<td>CHO-10</td>
<td></td>
<td>1.0</td>
<td>12</td>
<td>73.7</td>
<td>22 19-23</td>
<td>67% at 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese hamster V79</td>
<td></td>
<td>0.67</td>
<td>10</td>
<td>76.3</td>
<td>22 19-23</td>
<td>51% at 22</td>
<td>1.22</td>
<td>36.1-38.3</td>
</tr>
<tr>
<td>Kangaroo rat CCL56</td>
<td></td>
<td>1.53</td>
<td>31</td>
<td>62.3</td>
<td>14 12-16</td>
<td>47% at 14</td>
<td>2.13</td>
<td>35.7</td>
</tr>
<tr>
<td>Mouse LP59</td>
<td></td>
<td>1.41</td>
<td>22</td>
<td>69.0</td>
<td>40 45-58</td>
<td>18% at 50</td>
<td>2.52</td>
<td>37.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> For thermal resistance, one is most resistant and 6 is most sensitive.

<sup>b</sup> Volume of cell lines per volume of CHO cells.

<sup>c</sup> Data taken from Refs. 3 and 17.

<sup>d</sup> J. B. Mitchell, J. S. Bedford, and S. M. Bailey, manuscript in preparation.

DISCUSSION

The data from Chart 1 demonstrate that differences in culture media, possibly differences in sera, can result in differences in the cellular hyperthermic response. Such factors may account for some of the differences in cellular heat responses when the same cell lines are studied in several laboratories (16, 17, 23, 29, 36). However, the data from Charts 2 and 3 show that intrinsic differences in cellular heat responses do exist even when the culture techniques and treatment procedures are identical for all the cell lines. In general, the cells that are resistant to heat inactivation at 42.5° are also resistant at 45.5°; however, the order of sensitivity for some of the cell lines is different at 42.5 and 45.5°. This is especially obvious for the V79 cells, which are most sensitive at 45.5°, but are more resistant than 4 cell lines at 42.5°. Thus, differences in cellular heat sensitivity are also temperature dependent. Thermal tolerance, as observed by others (10, 34), for continuous heating at 41.5-42.5° is also observed for most of the cell lines after heating at 42.5° for about 4 hr, but occurs at different survival levels. However, tolerance is only transitory for rat kangaroo cells and is absent for mouse cells at 42.5°. Thus, for comparison of cellular thermal sensitivity, such factors as differences in media, pH (9, 10), and treatment temperatures should be considered.

It appears from the data presented in Table 1 that neither cellular heat sensitivity nor radiosensitivity is related to cell volume, cell population doubling times, plating efficiency, DNA content, or chromosome number. However, positive correlation does exist between the increase in cellular heat sensitivity and the decrease in body temperature of the species from which the cell line was derived. Also, the data in Chart 5 and Table 2 show that the X-ray sensitivity of all the cell lines can be enhanced by 1 hr of thermal treatment at 42.5° before X-irradiation. An increase in thermal radiosensitization, i.e., the thermal enhancement ratio, is related to an increase in cellular heat sensitivity. However, there is no correlation between differences in heat sensitiv-
with great caution. The clinical situation with humans should also be viewed together or separately. Variations in the heat response of cells to the 2 treatment modalities used to spend differently to the 2 treatment modalities used to 2 treatment modalities used to

Many investigations to evaluate the use of hyperthermia for cancer treatment involve in vivo studies carried out in mice, and our data show that of the 7 cell lines, the mouse LP59 cells have the greatest thermal sensitivity, and, unlike the other cell lines, do not display thermal tolerance at 42.5°. Thus, extrapolation of data from studies with mice to the clinical situation with humans should also be viewed with great caution.

Table 2
Survival data from Charts 4 and 5

<table>
<thead>
<tr>
<th></th>
<th>X-ray</th>
<th>Heat + X-ray</th>
<th>Survival 1 TER 1 hr at hr at 42.5° 42.5°+ X-ray DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0 (rads)</td>
<td>n</td>
<td>D0 (rads)</td>
</tr>
<tr>
<td>Pig kidney CCL33</td>
<td>148</td>
<td>3</td>
<td>149</td>
</tr>
<tr>
<td>Munțjac CCL157</td>
<td>128</td>
<td>5</td>
<td>96</td>
</tr>
<tr>
<td>HeLa S3</td>
<td>117</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>CHO-10</td>
<td>109</td>
<td>15</td>
<td>91</td>
</tr>
<tr>
<td>Chinese hamster</td>
<td>156</td>
<td>7</td>
<td>106</td>
</tr>
<tr>
<td>V79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat kangaroo</td>
<td>124</td>
<td>11</td>
<td>67</td>
</tr>
<tr>
<td>CCL56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse LP59</td>
<td>137</td>
<td>4</td>
<td>39</td>
</tr>
</tbody>
</table>

a Thermal enhancement ratios at S = 10^{-2} dose - X/dose - Δ X/normalized for heat killing.
b The dose-modifying factor at S = 10^{2} dose - V79/dose - cell lines.

REFERENCES

23. Leith, J. T., Miller, R. C., Gerber, E. W., and Boone, M. L. M. Hyperther-
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