Cellular Hypersensitivity to Neocarzinostatin in Ataxia-Telangiectasia
Skin Fibroblasts

Yosef Shiloh, Einat Tabor, and Yechiel Becker

INTRODUCTION

A-T is a human autosomal recessive disease featuring cerebellar degeneration, oculocutaneous telangiectases, immunodeficiency, cancer proneness, and radiation sensitivity (see Ref. 27 for review). Cultured cells from A-T patients show increased spontaneous chromosomal breakage and are hypersensitive to the clastogenic and cytotoxic effects of ionizing radiation and bleomycin (19, 27, 35) that produce mainly strand breaks and alkali-labile sites in the DNA (37, 39). The A-T fibroblast strains tested in our laboratory were found to be hypersensitive to the cytotoxic action of another DNA-breaking agent, the antitumor antibiotic NCS. This drug has been shown previously to inhibit DNA synthesis and to induce single- and double-stranded breaks in the DNA in vivo and in vitro (2, 3, 11, 16, 23, 28, 36).

MATERIALS AND METHODS

Cells. The fibroblast strains with local designation (Table 1) were all established at the Department of Human Genetics, Hebrew University-Hadassah Medical Center, Jerusalem, Israel, from skin biopsies donated voluntarily. Strain AT3BI was a generous gift from Dr. A. R. Lehmann, MRC Cell Mutation Unit, The University of Sussex, Brighton, England. The cells were maintained in Dulbecco’s modified Eagle’s medium (Grand Island Biological Co., Grand Island, N. Y.) containing 15% heat-inactivated fetal calf serum (Seralab, Crawley Down, West Sussex, England) and were tested at passage levels 6 to 13 (A-T strains) or 6 to 19 (normal strains), when serial passage of cells was made at a split ratio of 1:3.

Survival Experiments. Exponentially growing cultures were treated in phosphate-buffered saline [0.8% (w/v) NaCl:0.02% (w/v) KCl: 0.29% (w/v) Na2HPO4:0.02% (w/v) KH2PO4] with the desired concentrations of NCS for 1 hr at 37° [NCS produced by Kayaku Antibiotics Research Co., Ltd., Tokyo (Lot 730589), was a generous gift from Dr. B. Strauss, University of Chicago]. Following treatment, the cells were immediately trypsinized and seeded in appropriate dilutions in 5-cm tissue culture dishes (Nunc, Roskilde, Denmark) containing 5 ml of Ham’s F-10 medium (Grand Island Biological Co.) supplemented with 20% fetal calf serum. Four to 10 plates were seeded for each determination. Following incubation for 10 days, the cultures were stained with 0.2% crystal violet solution in 0.8% (w/v) NaCl, and colonies composed of 50 cells or more were counted under a dissecting microscope. The D0 values were calculated from the survival curves, using a linear regression program.

RESULTS

The D0 values obtained for each cell strain following treatment with NCS (Table 1) were consistent over repeated experiments. Eight A-T strains derived from donors belonging to 6 sibships showed a significantly higher sensitivity to cytotoxicity of NCS, their average D0 being 14.6 ng/ml, as compared to an average D0 of 37.9 ng/ml for the 3 normal strains. The D0 values of all the Jerusalem A-T strains were very close [15.0 ± 0.3 ng/ml (S.D.)]; however, strain AT3BI showed a somewhat higher sensitivity (D0, 11.5 ± 0.3 ng/ml). The D0 for the 2 heterozygous strains (26.4 ± 0.4 ng/ml) was close to the midpoint between normals and A-T homozygotes. An evident difference between the normal and the A-T homozygous strains was observed with regard to the slope and shoulder size of the survival curves (Chart 1), while the 2 heterozygous strains formed a third distinct curve.

DISCUSSION

The difference in sensitivity to NCS between normal and A-T fibroblasts resembles their different sensitivities to X-rays (1, 34, 40). In both cases, the D0 values obtained with A-T cells were roughly 2.5-fold smaller than those obtained with normal cells. Both the control and the A-T strains established in our institution constitute homogeneous groups with regard to NCS sensitivity, and the variation between strains in each group is relatively small (Table 1; Chart 1). It is of interest that strain AT3BI, which showed a somewhat higher sensitivity to NCS than did the Jerusalem A-T strains, was also found to be more sensitive to bleomycin than was another British A-T strain, AT5BI (19). The Jerusalem A-T strains are derived from patients of North African and Middle Eastern origin, and their ethnic extraction is probably entirely different from that of previously described A-T strains from Europe and North Amer-
Table 1

Description of sensitivity to NCS of human fibroblast strains

<table>
<thead>
<tr>
<th>Local designation</th>
<th>International designation for A-T cell lines</th>
<th>Age of donor (yr)</th>
<th>Sex</th>
<th>Country of origin</th>
<th>Colony-forming efficiency (%)</th>
<th>( D_0 ) (ng/ml) of NCS</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-107</td>
<td></td>
<td>28</td>
<td>M</td>
<td>Israel</td>
<td>31.9 ± 2.1 (^c)</td>
<td>38.6 ± 1.2</td>
<td>5</td>
</tr>
<tr>
<td>F-187</td>
<td></td>
<td>29</td>
<td>M</td>
<td>Israel</td>
<td>30.8 ± 2.3</td>
<td>37.1 ± 0.8</td>
<td>4</td>
</tr>
<tr>
<td>F-196</td>
<td></td>
<td>11</td>
<td>M</td>
<td>Israel</td>
<td>33.2 ± 1.5</td>
<td>30.0 ± 0.9</td>
<td>4</td>
</tr>
<tr>
<td>A-T homozygotes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>F-112</td>
<td>AT17UE-F</td>
<td>21</td>
<td>F</td>
<td>Morocco</td>
<td>29.8 ± 2.4</td>
<td>15.1 ± 0.1</td>
<td>2</td>
</tr>
<tr>
<td>F-113</td>
<td>AT18UE-F</td>
<td>18</td>
<td>F</td>
<td>Morocco</td>
<td>25.5 ± 1.2</td>
<td>14.7 ± 0.3</td>
<td>3</td>
</tr>
<tr>
<td>F-131</td>
<td>AT19UE-F</td>
<td>9</td>
<td>F</td>
<td>Morocco</td>
<td>18.1 ± 1.7</td>
<td>15.5 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>F-143</td>
<td>AT20UE-F</td>
<td>5</td>
<td>M</td>
<td>Iran</td>
<td>23.2 ± 2.0</td>
<td>15.0 ± 0.3</td>
<td>2</td>
</tr>
<tr>
<td>F-144</td>
<td>AT21UE-F</td>
<td>9</td>
<td>M</td>
<td>Iran</td>
<td>24.3 ± 1.8</td>
<td>15.3 ± 0.5</td>
<td>4</td>
</tr>
<tr>
<td>F-169</td>
<td>AT22UE-F</td>
<td>3</td>
<td>F</td>
<td>Israel (Arab)</td>
<td>22.8 ± 1.7</td>
<td>14.5 ± 0.4</td>
<td>2</td>
</tr>
<tr>
<td>F-182</td>
<td>AT23UE-F</td>
<td>14</td>
<td>M</td>
<td>Morocco</td>
<td>30.7 ± 2.0</td>
<td>15.2 ± 0.2</td>
<td>3</td>
</tr>
<tr>
<td>AT3Bl</td>
<td></td>
<td>8</td>
<td>M</td>
<td>England</td>
<td>11.2 ± 0.5</td>
<td>11.5 ± 0.3</td>
<td>2</td>
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<tr>
<td>A-T heterozygotes</td>
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<td></td>
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<td></td>
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<td>F-198</td>
<td>AT60UE-F</td>
<td>39</td>
<td>M</td>
<td>Morocco</td>
<td>29.0 ± 1.3</td>
<td>26.7 ± 0.4</td>
<td>3</td>
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<tr>
<td>F-218</td>
<td>AT61UE-F</td>
<td>48</td>
<td>M</td>
<td>Morocco</td>
<td>28.3 ± 1.8</td>
<td>27.2 ± 0.6</td>
<td>3</td>
</tr>
<tr>
<td>Group mean ± S.D.</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>37.9 ± 0.8</td>
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</table>

\(^a\) The uniform system for designation of A-T cell strains was agreed upon at the International Workshop on A-T held at MRC Cell Mutation Unit, The University of Sussex, Brighton, England, November 1980 (B. A. Bridges and D. G. Harnden, eds. Ataxia-Telangiectasia: A Cellular and Molecular Link Between Cancer, Neuropathology and Immune Deficiency. London: John Wiley and Sons, 1982).

\(^b\) Colony-forming efficiency was defined as the number of colonies obtained from untreated cells divided by the number of cells seeded. Mean values are given for A-T strains at passage levels 6 to 13. At higher passages, the colony-forming efficiency of A-T cells significantly decreased.

\(^c\) Mean ± S.D.

\(^d\) Mean ± S.D. not including AT3Bl: 15.0 ± 0.3 ng/ml.

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A shoulder merging into an exponential decline and are consistent for the same cell strains over repeated experiments, unlike the situation with bleomycin which yields survival curves characterized by a biphasic exponential part and variable results in different experiments, probably because of its sensitivity to a number of variables, such as pH variations, oxygen concentration, and the presence of reducing agents and trace metal contaminants (19, 35). NCS, therefore, may be a more convenient agent for use in laboratory diagnosis of A-T, especially when an X-ray machine is not available.

The 2 A-T heterozygous strains showed an intermediate sensitivity to NCS, as compared with that of the normal and A-T strains (Table 1; Chart 1). A-T heterozygotes constitute a cancer-prone population (31) but are otherwise healthy, and identification of these individuals may contribute to the understanding of the epidemiology of cancer. To date, there is no satisfactory laboratory method for A-T heterozygote detection. Paterson et al. (26) reported on intermediate hypersensitivity of several A-T heterozygous strains to hypoxic \(\gamma\)-irradiation; however, other heterozygous strains tested showed normal sensitivity. We are currently testing additional series of heterozygous strains, in order to see whether similar variability also applies to sensitivity to NCS.

NCS, an antitumor antibiotic (14) composed of an acidic peptide moiety (20) and a nonprotein chromophore (22), acts via the release of the chromophore, which in turn interacts with the DNA (17). As a result of NCS action, the DNA is degraded both in vitro (2, 36) and in vivo (2, 3, 23, 28). This degradation is coupled to the release of DNA bases, particularly thymine (11, 15), and to the production of apurinic-apyrimidines sites as intermediates (4). It has recently been shown that direct deoxyribose damage is involved in DNA strand scission by NCS (10). The hypersensitivity of A-T cells to NCS is additional evidence of the unusual response of A-T cells to DNA-breaking agents. However, cells from A-T patients have been shown to rejoin DNA breaks induced by ionizing radiation and bleomycin.
A-T cells and that this fraction is too small to be detected by forward to account for X-ray and bleomycin hypersensitivity in processes irradiated DNA in vivo. Another explanation put forward to account for X-ray and bleomycin hypersensitivity in deficient human cell lines. Nucleic Acid Res., 5: 463–473, 1978.


REFERENCES


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