Effects of the DNA Strand-cleaving Antitumor Agent, Streptonigrin, on Ataxia Telangiectasia Cells

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ABSTRACT

Cells from patients with ataxia telangiectasia (A-T) were shown to be more sensitive to streptonigrin than were cells from normal individuals. A linear dose-dependent cell survival was observed for both normal and A-T cells exposed to streptonigrin (up to 1.5 ng/ml) for 3 hr, with the A-T cells being about twice as sensitive as were the normal cells (D0 ~ 0.25 ng compared with D0 ~ 0.5 ng). The extreme toxicity of streptonigrin is also seen in the response of DNA synthesis which is inhibited sharply in both A-T and normal cells using doses of up to 125 ng/ml, although the effect was less pronounced in A-T cells. A greater amount of time was needed for recovery of DNA synthesis in normal cells compared with that of A-T cells. Finally, chromosomes from both A-T lymphocytes and fibroblasts show about a doubling of breakage rate following exposure to streptonigrin. The increased sensitivity of A-T cells to streptonigrin appears to be fairly comparable to the sensitivity to ionizing radiation, bleomycin, or neocarzinostatin and provides further evidence that perhaps A-T cells are deficient in some form of DNA strand repair.

INTRODUCTION

Cells from patients with the cancer-prone condition A-T have been shown to be unusually radiosensitive by both colony survival assays and chromatographic methods. DNA synthesis in A-T cells is far less inhibited following exposure to X-rays compared with that in normal control cells. A-T cells have also been shown to be unusually sensitive to the antitumor agent bleomycin, and most recently to neocarzinostatin. We report here that A-T cells are also unusually sensitive to the DNA strand-cleaving antitumor agent streptonigrin.

MATERIALS AND METHODS

Cells and Culture Conditions. Both normal control fibroblast strains (REE, BAK, JAC, BRO, HOW, POS, PEA, and BAR) and A-T fibroblasts (AT3BI, AT5BI, and AT7BI) were grown routinely in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Flow Laboratories), glutamine, penicillin (100 IU/ml), and streptomycin (100 µg/ml), and they were incubated at 37° in 5% CO2 in air.

Lymphocytes for chromosome preparations were obtained from 4 additional A-T patients (AT2BI, AT5BI, AT11BI, and AT19BI). Heparinized whole blood (0.4 ml) was cultured in 4.0 ml of Ham’s F-10 medium supplemented with 10% bovine serum, 1% phytohemagglutinin, penicillin, and streptomycin.

Streptonigrin was supplied by Dr. H. Wood, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. The drug was prepared for use by dissolving in 0.1 M Tris at 250 µg/ml and was stored in aliquots at -50° until required. For some chromosome preparations, the drug was dissolved in 4.8% sodium bicarbonate and used immediately.

Survival Curves. Different cell dilutions were seeded on to lethally irradiated feeder layers of the same cells (6 x 104 cells/9-cm dish irradiated with 35 gray 60Co γ-rays). After 24 hr, the normal medium was replaced by medium containing streptonigrin (0.25 to 1.5 ng/ml) for 3 hr. The drug was removed, the cells were washed with 0.9% NaCl solution, and fresh medium with 10% fetal calf serum was placed on the cells. Cells were left for 14 to 21 days in an incubator to form colonies with medium changing once a week. Colonies were stained with methylene blue.

Inhibition of DNA Synthesis. The response of DNA synthesis in A-T fibroblasts following streptonigrin exposure was checked using cell strains AT3BI and AT5BI. Cells were incubated for 24 hr at 37° in growth medium containing [2-3H]thymidine (0.01 µCi/ml; 54 mCi/mmol). This medium was removed and replaced by MEM (thymidine-free) supplemented with 10% fetal calf serum and 10% glutamine (2 mCi/ml) for 1 hr. Streptonigrin dissolved in 0.1 M Tris was added at various doses (6.25 to 125 ng/ml) for 1 hr at 37°. The streptonigrin-containing medium was removed, the cells were washed with fresh MEM, and more MEM was added to the cells for 30 min. DNA synthesis was measured by the addition of [methyl-3H]thymidine (10 µCi/ml; 48 Ci/mmol) for 20 min at 37°. This was removed, and the cells were washed in ice-cold Dulbecco A buffer before adding 1 ml of 20% trichloroacetic acid and 200 µl EDTA (0.02%). The cells were scraped off the dishes by using a rubber policeman and were left for 2 hr to allow for precipitation of the acid-insoluble material. This was collected on Whatman GF/C filters, and the filters were washed 3 times in 5% trichloroacetic acid/2% sodium pyrophosphate. The filters were dissolved in Instagel, and both 14C and 3H activities were counted.

For experiments in which the time of recovery of DNA synthesis was measured following streptonigrin treatment, AT5BI and normal POS or BRC cells were first labeled with [3H]thymidine as described above and were exposed to the drug (12.5 ng/ml) for 1 hr. The cells were washed, and fresh medium was put on the dishes. At different times (1 to 7 hr) after removal of streptonigrin, [3H]thymidine was added for 20 min in the same way as for dose-response curves, and both 14C and 3H activities were counted.

Chromosome Preparations. In lymphocyte cultures, streptonigrin made up in bicarbonate or Tris buffer was added at either 44 or 48 hr after culture initiation for 1 to 4 hr. Following exposure to the drug, cells were centrifuged, washed, and resuspended in growth medium. The cells were harvested at either 48 or 72 hr. Chromosome preparations were obtained from fibroblasts by subculturing the confluent monolayer to 2 x 106 cells/5-cm dish and treated at 44 hr after trypsinization with...
RESULTS

Streptonigrin is clearly an extremely toxic compound. Both normal and A-T fibroblasts showed a linear survival curve following streptonigrin treatment (Chart 1) with the normal showing a slight shoulder, although the A-T cells were about twice as sensitive to the drug as were normal cells (D0 for A-T cells ~0.25 and ~0.5 ng for normal controls). Strains AT3BI, AT5BI, and AT7BI appeared to have the same sensitivity to the drug.

The A-T fibroblast strains (AT5BI and AT7BI) used for survival curves were examined for their chromosomal sensitivity to streptonigrin. Following treatment with streptonigrin (50 ng/ml in bicarbonate) for 3 hr at 44 hr after subculturing, a doubling in the number of chromatid-type aberrations was seen in the A-T cells compared with that in the normals (Table 1). The frequency of fragments was also slightly higher in the A-T cells.

Lymphocyte chromosomes from short-term whole-blood cultures from 2 additional A-T patients (AT11BI and AT19BI) were treated just before harvest at 48 hr. Blood from patient AT11BI was treated 1 hr before harvest with streptonigrin (50 ng/ml in bicarbonate), and blood from patient AT19BI was treated 3 hr before harvest at 48 hr with streptonigrin (5 ng/ml in Tris buffer). In each case, the A-T blood showed about a 4-fold increase in chromatid-type damage. In the A-T lymphocytes, there was a small number of heavily damaged cells reminiscent of the situation following bleomycin treatment (28). Following exposure to streptonigrin at 48 hr (100 ng/ml in bicarbonate) for 1 hr and harvest at 72 hr, the levels of chromatid-type aberrations were fairly comparable in A-T cells and controls, although the level of fragments was slightly higher in the A-T cells (Table 2).

Streptonigrin is a very potent inhibitor of DNA synthesis in normal cells where a 125-ng/ml dose of the drug will reduce DNA synthesis to about 10% of the untreated control level in 1 hr. A-T cells are also very sensitive to streptonigrin although not to the same extent as are normals. At all doses, there is a clear and consistently higher level of DNA synthesis in A-T cells which is most noticeable at low doses (Chart 2). The dose of the drug required to reduce DNA synthesis to 50% of the untreated A-T sample is at least double the dose required for normal cells. The 2 A-T fibroblast lines examined (AT3BI and AT5BI) appear to have the same response to streptonigrin.

Following treatment of both normal and A-T cells (AT3BI) with streptonigrin (12.5 ng/ml), DNA synthesis in the normals fell to a minimum of about 60% of the untreated control, compared with about 85% in the A-T cell strain. Recovery of DNA synthesis to the control level in the normal cells took at least twice the time taken by the A-T cells (Chart 3). There is clearly a greatly enhanced level of DNA synthesis in the A-T cells after a 3-hr recovery as though there were stimulation of DNA synthesis. The reason for this is not understood at present.

DISCUSSION

A-T cells have previously been shown unequivocally to be unusually sensitive to ionizing radiation (6, 26, 27), bleomycin (4, 8, 14, 28), and neocarzinostatin (23, 24) as measured by several criteria. A linear response of survival was observed here with the dose of streptonigrin used. The differential between D0 values for A-T and normal cells was about 2-fold which is a little less than that for ionizing radiation (26) and bleomycin (28). There is no suggestion in the present results of a differential effect of the drug within the A-T strains examined even though at least 2 complementation groups may be represented (11, 20).

The precise mechanism of action of streptonigrin is unknown, although it has been suggested that streptonigrin reduced by NADH forms radicals, such as superoxide or hydroxyl, which produce the DNA damage (2, 13). Chromosomally, there is an increased sensitivity of A-T cells to streptonigrin as also seen for X-rays, bleomycin, talisomycin, and neocarzinostatin. When dissolved in 4.8% sodium bicarbonate, streptonigrin gradually breaks down. This effect probably explains the similar levels of

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Table 1

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>No. of cells analyzed</th>
<th>No. of fragments</th>
<th>No. of chromatid gaps</th>
<th>No. of chromatid breaks</th>
<th>No. of chromatid gaps</th>
<th>No. of chromatid interchanges</th>
<th>Chromatid damage/100 cells</th>
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<td>AT5BI</td>
<td>50</td>
<td>27</td>
<td>671</td>
<td>137</td>
<td>4</td>
<td>1</td>
<td>16.18</td>
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<tr>
<td>AT7BI</td>
<td>25</td>
<td>11</td>
<td>336</td>
<td>74</td>
<td>2</td>
<td>0</td>
<td>16.44</td>
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<tr>
<td>ConBAR</td>
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<td>3</td>
<td>313</td>
<td>63</td>
<td>3</td>
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<td>ConPEA</td>
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<td>208</td>
<td>41</td>
<td>1</td>
<td>0</td>
<td>8.30</td>
</tr>
</tbody>
</table>

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Chart 1. Effect of streptonigrin on the survival of A-T and normal fibroblast cell strains following simultaneous treatment for 3 hr. A-T cells: AT3BI (mean of 4 experiments); AT5BI (mean of 2 experiments); AT7BI (mean of 3 experiments). Normal cells: O, ConBAK (mean of 5 experiments); , ConREE (mean of 3 experiments); , ConBRO (result of one experiment). Bars, S.E.
Table 2

Chromosome analysis of A-T lymphocytes treated with streptonigrin

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of cells analyzed</th>
<th>No. of ring chromosomes/dicentric chromosomes</th>
<th>No. of fragments</th>
<th>No. of chromatid gaps</th>
<th>No. of chromatid breaks</th>
<th>No. of gaps</th>
<th>No. of chromatid interchanges</th>
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</thead>
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<tr>
<td>Streptonigrin (50 ng/ml in bicarbonate) for 1 hr before harvest at 48 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT11BI</td>
<td>27*</td>
<td>0(0)</td>
<td>3(2)</td>
<td>62(3)</td>
<td>30(1)</td>
<td>2(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Con 559</td>
<td>30</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptonigrin (5 ng/ml in Tris buffer) for 3 hr before harvest at 48 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT190BI</td>
<td>48</td>
<td>0(0)</td>
<td>5(2)</td>
<td>32(0)</td>
<td>7(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Con 1737</td>
<td>50</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptonigrin (100 ng/ml in bicarbonate) for 1 hr at 48 hr and recultured to 72 hr</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>AT2BI</td>
<td>51</td>
<td>6</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>24(1)</td>
<td>16(0)</td>
</tr>
<tr>
<td>AT8BI</td>
<td>30</td>
<td>4(6)</td>
<td>24(1)</td>
<td>13(2)</td>
<td>5(0)</td>
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<td>0</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Three additional cells with multiple chromosome damage.

Numbers in parentheses, number of spontaneously occurring aberrations in A-T cells.

Chart 2. Effect of increasing streptonigrin dose on DNA synthesis in both A-T and normal fibroblast cell strains. Fibroblasts were prelabeled with [3H]thymidine and were either left untreated or exposed for 1 hr to various concentrations of streptonigrin (6.25 to 125.0 ng/ml). Cells were then pulse-labeled for 20 min with [3H]thymidine, and the level of radioactivity in the acid-insoluble fraction was determined. Ordinates, 3H/14C ratio in treated versus untreated cells. A, AT3BI (4 experiments); •, AT5BI (mean of 3 experiments); ○, ConPOS (mean of 2 experiments); □, ConBRO (result of 1 experiment). Bars, S.E.

Chart 3. DNA synthesis following treatment of both an A-T and normal cell strains with streptonigrin (12.5 ng/ml) for 1 hr. At various times after drug treatment, cells were pulse-labeled with [3H]thymidine for 20 min and the acid-insoluble radioactivity was determined as in Chart 2. ●, AT5BI (mean of 3 experiments); ○, ConPOS (mean of 2 experiments); □, ConBRO (result of 1 experiment). Bars, S.E.
similar to the response seen following neocarzinostatin treatment of these cells, where again a biphasic response is seen, in which replication initiation is more inhibited than with bleomycin or ionizing radiation (24).

The types of response by A-T cells to a particular measure of sensitivity cannot be predicted by the type of response to another criterion of sensitivity. Therefore, survival may be linear or biphasic with dose, and DNA synthesis may or may not be biphasic with dose. For each agent, however, decreased survival in A-T cells is correlated with a lesser inhibition of DNA synthesis. The relationship between these 2 features is not understood at present. The fundamental defect in A-T cells may be an inability to repair some form of as yet unidentified DNA damage. The clearest evidence for this comes from the finding that A-T cells are unable to undergo potential lethal damage repair (5, 29) and more indirect evidence from chromosomal work suggesting the persistence of some type of unrepaired DNA strand breaks (25). Ionizing radiation, bleomycin, and neocarzinostatin are all able to produce DNA strand breaks. This is also true for streptonigrin and tends to lend support for the notion that perhaps there is a particular form of DNA strand break which, in A-T cells, is refractory to repair.

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REFERENCES


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