ABSTRACT

Cells from patients with ataxia telangiectasia (A-T) have been shown to be unusually radiosensitive in both colony survival assays and chromosomal methods. DNA synthesis in A-T cells is far less inhibited following exposure to X-rays compared with that in normal control cells (6, 10, 21). A-T cells have also been shown to be unusually sensitive to the antituor agent bleomycin (4, 8, 14, 28) and most recently to neocarzinostatin (23, 24) using broadly the same criteria. We report here that A-T cells are also unusually sensitive to the antituor antibiotic streptonigrin (NSC 45383). This is a known chromosomally radiomimetic drug capable of causing chromosome-type aberrations following G0 exposure of cells and chromatid-type aberrations following exposure to G2 (1, 9, 12, 22). Streptonigrin will inhibit DNA synthesis (16, 18, 19, 31) and chromosome-type aberrations following G0 exposure of cells and prevents DNA strand breakage (17, 19, 30). The mode of action of streptonigrin is not well understood, but a repeated suggestion is that the superoxide and/or hydroxyl radicals are the active species in producing DNA strand scissions (2).

INTRODUCTION

Cells from patients with the cancer-prone condition A-T have been shown to be unusually radiosensitive by both colony survival assays and chromosomal methods (16, 26, 27). DNA synthesis in A-T cells is far less inhibited following exposure to X-rays compared with that in normal control cells (5, 6, 10, 21). A-T cells have also been shown to be unusually sensitive to the antituor agent bleomycin (4, 8, 14, 28) and most recently to neocarzinostatin (23, 24) using broadly the same criteria. We report here that A-T cells are also unusually sensitive to the antituor antibiotic streptonigrin (NSC 45383). This is a known chromosomally radiomimetic drug capable of causing chromosome-type aberrations following G0 exposure of cells and chromatid-type aberrations following exposure to G2 (1, 9, 12, 22). Streptonigrin will inhibit DNA synthesis (16, 18, 19, 31) and produces DNA strand breakage (17, 19, 30). The mode of action of streptonigrin is not well understood, but a repeated suggestion is that the superoxide and/or hydroxyl radicals are the active species in producing DNA strand scissions (2).

MATERIALS AND METHODS

Cells and Culture Conditions. Both normal control fibroblast strains (REB, BAK, JAC, BRO, HOW, POS, PEA, and BAR) and A-T fibroblast strains (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°C in 5% CO₂ in air.

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 (D₅₀ ~ 0.25 ng compared with D₅₀ ~ 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 chromosomal methods (16, 26, 27). DNA synthesis in A-T cells is far less inhibited following exposure to X-rays compared with that in normal control cells (5, 6, 10, 21). A-T cells have also been shown to be unusually sensitive to the antituor agent bleomycin (4, 8, 14, 28) and most recently to neocarzinostatin (23, 24) using broadly the same criteria. We report here that A-T cells are also unusually sensitive to the antituor antibiotic streptonigrin (NSC 45383). This is a known chromosomally radiomimetic drug capable of causing chromosome-type aberrations following G0 exposure of cells and chromatid-type aberrations following exposure to G2 (1, 9, 12, 22). Streptonigrin will inhibit DNA synthesis (16, 18, 19, 31) and produces DNA strand breakage (17, 19, 30). The mode of action of streptonigrin is not well understood, but a repeated suggestion is that the superoxide and/or hydroxyl radicals are the active species in producing DNA strand scissions (2).

MATERIALS AND METHODS

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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

---

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 interchanges</th>
<th>Chromatid damage/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT5BI</td>
<td>50</td>
<td>27</td>
<td>671</td>
<td>137</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>AT7BI</td>
<td>25</td>
<td>11</td>
<td>336</td>
<td>74</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>ConBAR</td>
<td>50</td>
<td>3</td>
<td>313</td>
<td>63</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ConPEA</td>
<td>30</td>
<td>3</td>
<td>208</td>
<td>41</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

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* Streptonigrin Sensitivity of A-T Cells

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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>
</tr>
</thead>
<tbody>
<tr>
<td>Streptonigrin (50 ng/ml in bicarbonate) for 1 hr before harvest at 48 hr</td>
<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>
</tr>
<tr>
<td></td>
<td>Con 559</td>
<td>30</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Streptonigrin (5 ng/ml in Tris buffer) for 3 hr before harvest at 48 hr</td>
<td>AT19BI</td>
<td>48</td>
<td>0(0)</td>
<td>5(2)</td>
<td>32(0)</td>
<td>7(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>Con 1737</td>
<td>50</td>
<td>1</td>
<td>0</td>
<td>7</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>
<td>AT2BI</td>
<td>516</td>
<td>0(4)</td>
<td>24(1)</td>
<td>13(2)</td>
<td>5(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>AT8BI</td>
<td>30</td>
<td>4(6)</td>
<td>16(0)</td>
<td>4(0)</td>
<td>3(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>Con 516</td>
<td>30</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. Ordinate, [3H]/[14C] ratio in treated versus untreated cells. A, AT3BI (4 experiments); •, AT5BI (5 experiments); D, mean of 5 experiments with 4 different control cell strains (ConHOW, ConJAC, ConBRO, ConBAK).

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.

Chromosome aberrations caused by different doses of streptonigrin dissolved in bicarbonate and Tris buffer, respectively. Overall, however, the level of chromosomal sensitivity is 2- to 4-fold greater than that in normals depending on the time of exposure to the drug. As with bleomycin (28), the damage is wholly chromatid-type when the cells are exposed shortly before harvesting.

Following treatment of A-T cells with bleomycin or X-rays, there is only a slight inhibition of DNA synthesis (5-7, 10, 21). However, in normal cells following exposure to bleomycin or X-rays, DNA synthesis decreases in a biphasic manner with a very sensitive initial component followed by a less sensitive component (21). A-T cells show an absence of the initial sensitive component due to the almost total lack of inhibition of replicon initiation (7, 21). Following exposure of A-T cells to streptonigrin, DNA synthesis decreases in a biphasic manner as in normal cells (Chart 2), although not at the same initial rate and not to the same level. The response of DNA synthesis in A-T cells following streptonigrin treatment is clearly different from that seen following treatment with bleomycin or ionizing radiation. It is, however,
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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