Induction of Glucocorticoid-resistant Variants in a Murine Thymoma Line by Antitumor Drugs

Marianne Huet-Minkowski,2 Judith C. Gasson,3 and Suzanne Bourgeois4

ABSTRACT

Several antitumor drugs are shown to be mutagenic in murine thymoma lines: mitomycin C, bleomycin, streptonigrin, Colcemid, and BD40, an analog of ellipticine. Using conditions yielding 3 to 40% cell survival, all five drugs tested increase the frequency of glucocorticoid-resistant variants. Mitomycin C is as efficient as the classical alkylating agents N-methyl-N'-nitro-N-nitrosoguanidine and ethyl methanesulfonate. The other drugs, previously untested for mutagenic activity on mammalian cells, are weak mutagens yielding variants at frequencies 1 to 2 orders of magnitude lower than the alkylating agents. All 152 variants obtained result from defects in the glucocorticoid receptor. Variants induced by mitomycin C, streptonigrin, Colcemid, and BD40 have very reduced receptor activity, as measured by dexamethasone binding. In contrast, bleomycin or the combination of mitomycin C and dexamethasone induce a majority of variants having dexamethasone-binding activity comparable to the parental line. However, assays of nuclear transfer capacity and genetic complementation show that these receptors are nonfunctional and may result from point mutations in the gene encoding the glucocorticoid receptor. This study suggests that, in combination therapies, antitumor drugs might induce glucocorticoid-resistant lymphoid cell variants that could be selected by the hormone.

INTRODUCTION

The lymphocytolytic effect of glucocorticoids, allowing selection for resistant variants unresponsive to the hormone, is an attractive system to study the mode of action of steroid hormones. The murine lymphoma line S49 was first used extensively for such studies, and a large number of S49 variants resistant to dexamethasone have been isolated and characterized (19, 22, 23, 26). All dexamethasone-resistant S49 variants were found to result from defects in the glucocorticoid receptor. This lymphoma line was then shown to be functionally hemizygous for the gene, c, coding for the receptor, thus favoring the appearance of variants at the haploid r locus (r+ /r−). Another murine lymphoid line, the thymoma WEHI 7 (W7), was found recently to be homozygous for the r locus (r+/r+) and to be inducible for such studies, and a large number of W7 variants resistant to dexamethasone have been isolated and characterized (9). On the other hand, a variety of compounds, and especially some antibiotics used in cancer chemotherapy, are known to interfere with DNA synthesis through covalent and noncovalent binding to DNA (mitomycin C and streptonigrin), removal of bases from DNA (bleomycin) or intercalation (ellipticines), producing single- or double-strand breakage. In addition, the induction of chromosomal aberrations by mitomycin C is enhanced by caffeine which is known to inhibit postreplication repair mechanisms in mammalian cells (5, 15) and to have a suppressing effect on a prereplication repair mechanism in human peripheral lymphocytes after mitomycin C treatment (14).

The ability of these compounds to induce transmissible alterations in the genetic material of mammalian cells has only been demonstrated for mitomycin C [reviewed by Vig (24)], which acts by monofunctional alkylation (17) but differs from other alkylating agents, i.e., EMS, in the type of mutations induced. Caffeine was shown in some cases to potentiate the mutagenic activity of alkylating agents (21). Streptonigrin was shown to be a mutagen similar to UV in an ascomycete (27), while bleomycin appears to have a weak mutagenic and antimutagenic effect on Saccharomyces cerevisiae (11). The mutagenic effects on mammalian cells of streptonigrin and bleomycin are unknown, but these antitumor drugs give a negative result in the Ames test. BD40 is a newly synthesized product structurally similar to ellipticines; it appears to be a very potent and specific antitumor drug and was also found to be nonmutagenic in the Ames test (6, 7, 16). Lastly, Colcemid has never having normal receptor, would help to identify the unknown steps following the interaction of the receptor-steroid complex with the nucleus and leading to cell death.

Since dexamethasone-resistant variants of W7 arose spontaneously with a very low frequency (<10−10), mutagens had to be used. W7 mutagenesis was first performed with classical point mutagens, MNNG6 and EMS, and by UV irradiation, but again all the dexamethasone-resistant variants obtained were characterized as having defects at the receptor level (3). Assuming that nonreceptor variants exist but might be much less frequent than receptor variants, we turned to treatments known to induce large deletions and chromosomal rearrangements or even the elimination of an entire chromosome. The rationale of this approach is that extensive deletions or monosomy would increase the probability of producing cells haploid for an unknown gene playing a role in the cytolytic response to glucocorticoids. Such cells should give rise to dexamethasone-resistant variants resulting from defects in that unknown function with a much higher frequency than the parental line.

The elimination of one or 2 chromosomes, without chromosome rearrangement, was shown to be induced by treatment with Colcemid at low concentrations (9). On the other hand, a variety of compounds, and especially some antibiotics used in cancer chemotherapy, are known to interfere with DNA synthesis through covalent and noncovalent binding to DNA (mitomycin C and streptonigrin), removal of bases from DNA (bleomycin) or intercalation (ellipticines), producing single- or double-strand breakage. In addition, the induction of chromosomal aberrations by mitomycin C is enhanced by caffeine which is known to inhibit postreplication repair mechanisms in mammalian cells (5, 15) and to have a suppressing effect on a prereplication repair mechanism in human peripheral lymphocytes after mitomycin C treatment (14).

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4 Recipient of a grant from the Whitehall Foundation. To whom requests for reprints should be addressed.
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been shown to be a mutagen.

The results presented here demonstrate the mutagenic effect of these compounds on mouse lymphoid cell lines. These drugs are shown to increase the frequency of glucocorticoid-resistant variants, and the variants obtained are characterized.

MATERIALS AND METHODS

Chemicals. Bleomoxane (1 unit/mg), a mixture of A and B group bleomycines, was a generous gift from Bristol Laboratories (Syracuse, N.Y.); streptonigrin (NSC 45383) was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md.); Colcemid was purchased from Calbiochem-Behring Corp. (San Diego, Calif.); BD40, a 10-(γ-dieethylaminoethylpnaminolo)-6-methyl-5H-pyrido(3,4:4,5)pyrrolo(2,3-g)isoquinoline, synthesized by Ducrocq et al. (10), was a generous gift from Dr. J.C. Chermann (Institut Pasteur, France); mitomycin C, caffeine, and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, Mo.); [1,2-3H]dexamethasone (specific activity, 20 to 30 Ci/mmol) was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.).

Cell Lines. WEHI-7 (W7) arose as a thymoma in a female BALB/c mouse after X-irradiation; it was established in tissue culture and cloned by Harris et al. (12). We used derivatives of this cell line resistant to 6-thioguanine (W7TG) or 5-bromo-2'-deoxyuridine (W7TB). The presence of these markers allows the selection of cell hybrids and does not affect the response of these cell lines to dexamethasone (1). MS1, a spontaneous variant of W7TB, is sensitive to high concentrations of dexamethasone (10⁻⁸ to 10⁻⁴ M) but was selected as partially resistant to low concentrations of the steroid (5 × 10⁻⁸ M). The MS1 line is functionally hemizygous for the glucocorticoid receptor gene (r⁺/r⁻), and, therefore, gives rise spontaneously at high frequency to resistant variants resistant to high concentrations of dexamethasone (1).

Cell Growth. Cells were grown in suspension in Dublecco's modified Eagle's medium containing additional glucose (117 μg/ml) and 10% fetal calf serum. The 37°C incubator had a humidified atmosphere of 13% CO₂ and 87% air. One-liter cultures were grown in glass roller bottles, saturated with the air-CO₂ mixture, after cell inoculation. Living cell number was determined by the trypan blue exclusion technique.

Mutagenic Treatments and Selection for Resistance to Dexamethasone. Exponentially growing cells, at a density of 1 to 2 × 10⁶ cells/ml, were treated in fresh medium with the desired mutagen for the specified time. The treated cells were then washed free of the drug, resuspended in fresh medium, and grown as long as necessary for the surviving cells to resume exponential growth and to allow expression of resistance to dexamethasone. The growth of the cells was followed daily, and the selection with dexamethasone was begun when the cell number had increased for at least 2 days.

Two different protocols were followed to select the dexamethasone-resistant variants of the W7 line after recovery of the cells from the mutagenic treatment. After the mitomycin C and the streptonigrin treatments, 1-ml aliquots of 2 to 5 × 10⁵ cells/ml were treated in fresh medium with the desired mutagen for the specified time. The treated cells were then washed free of the drug, resuspended in fresh medium, and grown as long as necessary for the surviving cells to resume exponential growth and to allow expression of resistance to dexamethasone. The growth of the cells was followed daily, and the selection with dexamethasone was begun when the cell number had increased for at least 2 days.

The colony-forming efficiency of the dexamethasone-resistant cells was approximately 100%. When the total amount of cells treated with bleomycin, BD40, or Colcemid was higher than 10⁹, another protocol was used; the distribution in trays was performed after about 50 to 90% of the sensitive parental cells had been killed by incubation of the whole culture with 10⁻⁸ M dexamethasone. This reduces considerably the number of trays that have to be examined. However, the second protocol results in a predictable increase in the frequency of resistant variants obtained, because the variants undergo approximately 2 dou-
An exponentially growing culture of MS1 cells was treated for 22 hr with the indicated concentration of bleomycin, as described in Materials and Methods. After removal of the drug, exponential growth was allowed to resume, as illustrated in Chart 1. For selection of variants, the 2 protocols described in Materials and Methods were compared: direct plating in the presence of 10^{-6} M dexamethasone (Protocol 1) and plating after killing of approximately 50% of the parental cells by addition of 10^{-8} M dexamethasone to the growing culture (Protocol 2).

<table>
<thead>
<tr>
<th>Bleomycin (µg/ml)</th>
<th>% of survival</th>
<th>No. of cells in sample*</th>
<th>No. of resistant variants</th>
<th>Frequency of resistance</th>
<th>Fold increase over control</th>
<th>No. of resistant variants</th>
<th>Frequency of resistance</th>
<th>Fold increase over control</th>
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<tr>
<td>0</td>
<td>100</td>
<td>7.9 x 10^6</td>
<td>38</td>
<td>4.8 x 10^{-8}</td>
<td>1.0</td>
<td>109</td>
<td>1.4 x 10^{-8}</td>
<td>1.0</td>
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<td>10</td>
<td>5.0</td>
<td>5.8 x 10^6</td>
<td>129</td>
<td>2.3 x 10^{-8}</td>
<td>4.8</td>
<td>494</td>
<td>8.8 x 10^{-8}</td>
<td>6.3</td>
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<tr>
<td>30</td>
<td>0.9</td>
<td>2.0 x 10^6</td>
<td>76</td>
<td>3.8 x 10^{-8}</td>
<td>7.9</td>
<td>323</td>
<td>1.6 x 10^{-8}</td>
<td>11.4</td>
</tr>
<tr>
<td>50</td>
<td>&lt;0.5</td>
<td>9.5 x 10^5</td>
<td>66</td>
<td>6.9 x 10^{-8}</td>
<td>14.4</td>
<td>172</td>
<td>1.8 x 10^{-8}</td>
<td>12.8</td>
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<td>70</td>
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<tr>
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</tr>
</tbody>
</table>

* Total number of living cells present in the sample at the time of the addition of 10^{-8} M dexamethasone.

The first 2 columns in Table 1 show the toxicity of various doses of bleomycin, ranging from 10 to 100 µg/ml, on the MS1 line. Chart 2d compares the toxicity of bleomycin on the MS1 cell and W7 lines; the W7 line was found to be somewhat more resistant to bleomycin than the MS1 line, as well as to the other drugs tested (data not shown). This difference in sensitivity between the 2 lines was taken into account when experimental conditions, determined for MS1 treatment, were adjusted for the W7 line.

Chart 2, a, b, and c, show the dose-dependent toxicity of BD40, streptonigrin, and Colcemid on the W7 line. In the case of BD40 (Chart 2a) and streptonigrin (Chart 2b), the toxicity is also shown to be dependent on the length of the treatment. These toxicity tests enabled us to choose sublethal conditions of treatment to investigate the mutagenic effects of these various drugs.

**Mutagenic Efficiency of Various Drugs.** Table 1 (Columns 3 to 9) shows the dose-dependent increase in the frequency of induction of dexamethasone-resistant variants of the MS1 line by bleomycin. The maximum mutagenic effect of bleomycin was observed at 50 µg/ml, a dose resulting in 0.4% cell survival. Treatments by higher doses of bleomycin, 70 and 100 µg/ml, did not yield any variants because they were essentially lethal. Table 1 also compares the results obtained by using the 2 different protocols described in Materials and Methods for the selection of the dexamethasone-resistant variants. As expected, Protocol 2 results in apparent frequencies 2- to 4-fold higher than Protocol 1, due to one to 2 doublings of the resistant variants in the presence of dexamethasone before plating. However, the increase in frequencies over the untreated control (no bleomycin) is similar for both protocols.

Table 2 shows the efficiency of the different drugs tested in inducing dexamethasone-resistant variants of the W7 line, as compared to the spontaneous frequency and to the classical mutagens used in a previous study (3). The spontaneous frequency cannot be ascertained precisely because no variant was ever derived from 8.1 x 10^9 cells; therefore, this frequency must be <1.2 x 10^{-10}. Mitomycin C yielded variants at a frequency of the order of 10^{-7}, comparable to the mutagenic efficiency of MNNG, EMS, and UV irradiation. The 1-hr mitomycin C treatment was done in the presence and absence of 10^{-6} M dexamethasone to test the possibility that the induction of dexamethasone-responsive genes might make them more susceptible to the mutagenic treatment. This phenomenon has been observed in Escherichia coli, where an increased frequency of mutations in the β-galactosidase gene was obtained when mutagenic treatments were applied in the presence of an inducer of the lactose operon (4, 13). However, the presence of dexamethasone during the mitomycin C treatment did not increase the frequency of resistant variants, although, as will be described in the next section, the nature of the variants obtained appears to have been affected by the presence of the steroid.

Bleomycin, streptonigrin, BD40, and Colcemid are all mutagens in this system, but they appear less efficient than mitomycin C in that they increase the frequency of resistance only to 10^{-8} to 10^{-6}. While bleomycin and BD40 had not been shown previously to be mutagens, streptonigrin was found to be as efficient as UV irradiation in an ascomycete (27) but is only a weak mutagen in our animal cell system.

**Characterization of Dexamethasone-resistant Variants.** All the W7 dexamethasone-resistant derivatives that we obtained were assayed for their glucocorticoid receptor content by measuring binding of dexamethasone. Variants found to con-
The values obtained for the nuclear transfer capacity of receptors from 2 or 3 treatments in the presence of dexamethasone, followed by caffeine [(MC + Dex) or, mitomycin C followed by caffeine (MC + C)] (2 experiments); d, mitomycin C variants tested are given at the top of each panel, a, Colcemid (Colc); b, BO40; c, mitomycin C followed by caffeine (MC + C) (2 experiments); d, mitomycin C treatment in the presence of dexamethasone, followed by caffeine [(MC + Dex) + C] (3 experiments); e, bleomycin (Ble) (2 experiments).

Table 2

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Dose (µg/ml)</th>
<th>Time of treatment (hr)</th>
<th>No. of cells in sample</th>
<th>% of survival</th>
<th>No. of variants in sample</th>
<th>Frequency of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
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<tr>
<td>MNNG</td>
<td>1.25</td>
<td>2</td>
<td>8.1 x 10^6</td>
<td>5–10^4</td>
<td>0</td>
<td>&lt;1.2 x 10^-10</td>
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<tr>
<td>EMS</td>
<td>530</td>
<td>18</td>
<td>2.6 x 10^6</td>
<td>5–10^4</td>
<td>136</td>
<td>5.2 x 10^-1</td>
</tr>
<tr>
<td>UV</td>
<td></td>
<td></td>
<td>1.7 x 10^6</td>
<td>5–10^4</td>
<td>42</td>
<td>2.5 x 10^-1</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>0.5 or 1d</td>
<td>1</td>
<td>7.4 x 10^4</td>
<td>5</td>
<td>77</td>
<td>1 x 10^-7</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>0.5 or 1d</td>
<td>1</td>
<td>6.1 x 10^4</td>
<td>5</td>
<td>44</td>
<td>7.2 x 10^-4</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>47</td>
<td>22</td>
<td>1.4 x 10^4</td>
<td>10–15^5</td>
<td>49</td>
<td>3.5 x 10^-4</td>
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<tr>
<td>Streptonigrin</td>
<td>0.1</td>
<td>5</td>
<td>3.2 x 10^4</td>
<td>2</td>
<td>4</td>
<td>1.2 x 10^-3</td>
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<tr>
<td>BD40</td>
<td>0.03</td>
<td>2</td>
<td>4.3 x 10^4</td>
<td>3–10^4</td>
<td>8</td>
<td>1.8 x 10^-3</td>
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<tr>
<td>Colcemid</td>
<td>0.01</td>
<td>22</td>
<td>5.6 x 10^6</td>
<td>40</td>
<td>6</td>
<td>1 x 10^-4</td>
</tr>
</tbody>
</table>

Legend:
- Colcemid (Colc)
- BD40 (8)
- MC + C (53)
- (MC+Dex)+C(4)
- Bleomycin (Ble (44))

Induction of Glucocorticoid Resistance by Antitumor Drugs

Chart 3. Types of dexamethasone-resistant (DEX+) variants derived from the W7 line. Variants were selected as described in "Materials and Methods." The results obtained for the binding of dexamethasone (DEX BINDING) are expressed as the percentage of the binding observed in the parental line, and cell variants are classified according to their receptor content (abscissa). The ordinate shows the percentage of all variants belonging to each class defined on the abscissa. The results obtained with dexamethasone, as well as streptonigrin (not shown), gave rise essentially to variants having a very reduced amount of receptor, less than 25% of the parental line (Chart 3a, b, and c). These results are similar to the results obtained previously with classical mutagens (EMS, MNNG, or UV irradiation) (3). The distributions of variants obtained after mitomycin C treatment in the presence of dexamethasone (Chart 3d) and, more strikingly, after bleomycin mutagenesis (Chart 3e) are clearly different from the distributions obtained using the other treatments. The majority of the variants have greater than 25%, and some have the same amount of receptor as the parental line.

The variants containing a high amount of receptor (>50% of parent) were analyzed further to determine whether their receptor was functional or defective and could account for the resistant phenotype. By both biochemical and genetic criteria, we were able to demonstrate that the receptor of these variants was defective. The results of nuclear transfer assays are shown in Chart 3, d and e; all of the variants tested had only 24 to 50% of the parental capacity to translocate the receptor-steroid complex into the nucleus. Thus, these variants appear to be of the "nuclear transfer-defective" (nt-) type described previously (3).

Genetic complementation analysis was performed on 2 bleomycin-induced variants and on one variant induced by mitomycin C (Table 3). These variants were fused with well-characterized nt- variants of the S49 cell line, and the hybrids obtained were tested for dexamethasone resistance. If the receptor of these variants was functional, complementation would be observed; i.e., the hybrids would be dexamethasone sensitive. The S49 dexamethasone-resistant variants used in these fusions (variants 81, 66, and 222) have been shown elsewhere (18, 19) to result from receptor defects. Table 3 (Line 1) illustrates that such receptor defects are recessive in fusions with a parental cell (r+/r+). Lines 2 and 3 show that, as expected, the bleomycin-induced variant B101 containing a low amount of receptor does not complement the receptor defects of variants 81 and 66. The bleomycin-induced variant B20 and the mitomycin C-induced variant MC13, both containing a high amount of receptor (104 and 78% of the parental line, respectively), do not complement the receptor defect of variant 222 (see Table 3, Lines 4 and 5). Therefore, the receptors of variants B20 and MC13 are nonfunctional.

Another question that was addressed is whether drug-induced dexamethasone-resistant variants had developed any resistance to the drug used for mutagenesis. The bleomycin-induced variant B20 was as sensitive as the parent W7TB to 47 µg bleomycin per ml, and a mitomycin C-induced variant...
the alkylating agents (Table 2). These compounds are, indeed, although less efficient than mitomycin C in that they induced those conditions, all 4 drugs were found to be mutagenic, survival in the case of the very toxic drug Colcemid. Using define conditions of treatment resulting in 3 to 15% cell survival was determined (Chart 2). These measurements allowed us to
turally similar to ellipticines (7). BD40, which is under clinical investigation, appears to be much more selective and efficient in its killing activity on target tumor cells than ellipticines (7).

Vig (24). We observed that mitomycin C is as powerful a mutagen as MNNG and EMS for the W7 cell line, probably because of the alkylating properties of this drug (17). The other antitumor drugs used at which a considerable percentage of the population survived.

The nature of the glucocorticoid receptor defects observed varies with the mutagenic treatment. The vast majority of the variants induced by Colcemid, BD40, streptonigrin, or mitomycin C contained very reduced amounts of receptor, as measured by the dexamethasone-binding assay. These receptor defects probably result from deletions of the genetic material or other events leading to total loss or drastic reduction of receptor activity. In contrast, a high proportion of the variants induced by bleomycin, streptonigrin, and BD40 may be due to the fact that the extensive chromosome damage induced by these drugs is mostly lethal, even at the low concentrations used at which a considerable percentage of the population survived.

The high incidence of putative point mutations induced by bleomycin indicates that this compound acts differently from the other antitumor drugs tested. This result is surprising because bleomycin is known to induce single-stranded, as well as double-stranded, breaks resulting in deletions and extensive chromosome rearrangements that would be expected to inactive the r gene. Such extensive DNA damage must be mostly lethal, and therefore, the only DNA damages detected as receptor defects would be point mutations resulting from repair of single-stranded breaks by an error-prone repair mechanism. The contribution of DNA repair mechanisms to the mutagenic

(C23) remained as sensitive as W7TB to 1 µg mitomycin C per ml (data not shown). Therefore, we found no evidence for cross-resistance between the hormone and these antitumor drugs.

DISCUSSION

This study demonstrates the mutagenic activity, on murine lymphoid cell lines, of several drugs used in cancer chemotherapy. The drugs tested increase the frequency of appearance of glucocorticoid-resistant variants of the W7 and MS1 lines. Induction of glucocorticoid resistance in these lines had been shown previously to result from bona fide genetic events (1) which can also be induced by classical mutagens (3). The resistant phenotype of variants induced by MNNG, EMS, or UV was shown to be due to mutations in the gene, r, encoding the glucocorticoid receptor and to lead to measurable alterations in the properties of that protein (3). As expected from the genetic nature of these events, the frequency of resistant variants was found to be dependent on the ploidy of the r gene (1). The resistant variants induced by antitumor drugs and described in this study are shown to be of the same type as those induced by classical mutagens in that they also result from defects in the glucocorticoid receptor (Chart 3).

Of the 5 compounds tested in our system, only mitomycin C was known to be mutagenic for mammalian cells [reviewed by Vig (24)]. We observed that mitomycin C is as powerful a mutagen as MNNG and EMS for the W7 cell line, probably because of the alkylating properties of this drug (17). The other drugs tested were bleomycin, streptonigrin, Colcemid, and a newly synthesized compound, BD40, a dipyrindolindole structurally similar to ellipticines (7). BD40, which is under clinical investigation, appears to be much more selective and efficient in its killing activity on target tumor cells than ellipticines (7).

Since these 4 drugs had never been tested as mutagens on mammalian cells, their dose-dependent toxicity for the W7 line was determined (Chart 2). These measurements allowed us to define conditions of treatment resulting in 3 to 15% cell survival in the case of bleomycin, streptonigrin, and BD40 and 40% survival in the case of the very toxic drug Colcemid. Using those conditions, all 4 drugs were found to be mutagenic, although less efficient than mitomycin C in that they induced variants at frequencies 1 to 2 orders of magnitude lower than the alkylating agents (Table 2). These compounds are, indeed, known to act on DNA by different mechanisms producing single- or double-stranded breaks or, in the case of Colcemid, to induce the elimination of chromosome(s). Since Colcemid is known, at the concentrations used, to produce monosomic lines which are quite unstable (9), it is conceivable that some of the Colcemid-induced variants were lost during early passages of the treated cultures. The low frequencies of variants induced by bleomycin, streptonigrin, and BD40 may be due to the fact that the extensive chromosome damage induced by these drugs is mostly lethal, even at the low concentrations used at which a considerable percentage of the population survived.

The contribution of DNA repair mechanisms to the mutagenic

<table>
<thead>
<tr>
<th>Fusion Clone</th>
<th>% of parental nuclear transfer</th>
<th>Clones</th>
<th>% of parental nuclear transfer</th>
<th>No. of hybrids tested</th>
<th>Hybrids</th>
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<tbody>
<tr>
<td>BrdUrd resistant to 5-bromo-2'-deoxyuridine; TG resistant to thioguanine; ND, not done.</td>
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<td></td>
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<tr>
<td>Fusion described elsewhere (18), shown for comparison.</td>
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<tr>
<td>B101 is a bleomycin-induced, dexamethasone-resistant variant of line W7TG.</td>
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<td>B20 is a dexamethasone-resistant variant of line W7TB.</td>
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<tr>
<td>MC13 is a dexamethasone-resistant variant of line W7TB induced by treatment with mitomycin C in the presence of dexamethasone.</td>
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Table 3

Complementation analysis of bleomycin- and mitomycin C-induced variants

<table>
<thead>
<tr>
<th>Fusion Clone</th>
<th>% of parental nuclear transfer</th>
<th>Clones</th>
<th>% of parental nuclear transfer</th>
<th>No. of hybrids tested</th>
<th>Hybrids</th>
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<tbody>
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<td>BrdUrd resistant to 5-bromo-2'-deoxyuridine; TG resistant to thioguanine; ND, not done.</td>
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<tr>
<td>B101 is a bleomycin-induced, dexamethasone-resistant variant of line W7TG.</td>
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<td>B20 is a dexamethasone-resistant variant of line W7TB.</td>
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<td>MC13 is a dexamethasone-resistant variant of line W7TB induced by treatment with mitomycin C in the presence of dexamethasone.</td>
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effects observed cannot, however, be ascertained in these cells because nothing is known about their DNA repair activities. The high incidence of nuclear transfer-defective variants obtained makes bleomycin the mutagen of choice to induce these interesting receptor alterations observed rarely with classical mutagens.

An important and puzzling conclusion of this study is that all of the 152 variants examined result from receptor defects, similar to those demonstrated in 127 variants induced by classical mutagens and described previously (3). This study, therefore, brings to a total of approximately 300 the dexamethasone-resistant variants induced by a wide variety of mutagens and derived from the W7 line which is known to be diploid for the glucocorticoid receptor gene (r+/r+) (1). If a function other than the glucocorticoid receptor is involved in the cytolytic response, one might have expected to obtain variants having normal receptor but resistant to the steroid as the result of a defect in an as yet unknown function. The fact that no variant with normal receptor was observed amongst the approximately 300 variants examined must be considered as highly significant and deserves some comments.

At least 4 possible explanations for that observation must be considered. First, it is conceivable that mutations in a gene(s) other than r+, playing a role in the response, are at least 300-fold less frequent than in the r+ gene, either because of a smaller size of the locus involved or its location in the genome. Some of the mutagens used, in particular streptonigrin (8), bleomycin [reviewed by Vig (24)], and Colcemid (9) appear to have a nonrandom effect on chromosomes, affecting particular chromosomes preferentially. A second possibility is that other function(s) involved in the lytic response are vital to the cell and that defects in these functions are, therefore, lethal. It is also conceivable that 2 (or more) independent lytic pathways are induced following the interaction of the receptor-steroid complex with the nucleus. In that case, at least 2 functions would have to be inactivated simultaneously by mutations, a low-probability event. Finally, the possibility should be kept in mind that the receptor-steroid complex directly triggers the lytic process; i.e., no other function is involved and no new type of variant can be expected. This appears, however, unlikely in view of recent results obtained in our laboratory. A new type of W7 variants has been isolated that is supersensitive, rather than resistant, to glucocorticoids, while having normal receptor. Obviously, more work needs to be done to elucidate the mechanism of glucocorticoid-induced lysis and to account for the fact that all resistant variants examined so far have receptor defects.

In view of the known strong correlation between the mutagenic and carcinogenic properties of chemicals, it is worth examining the induction of genetic variations in mammalian cells by drugs used in cancer chemotherapy, since some cytotoxic drugs may be responsible for the appearance of secondary cancers. The carcinogenic properties of many antitumor agents have been demonstrated (see, e.g., Ref. 25). Some drugs, such as mitomycin C, were found to induce cancer at the site of injection, others had a broad spectrum of carcinogenicity affecting a variety of organs, while several agents led to tumors in the hematopoietic and lymphatic system. Of the drugs tested in the present study, bleomycin is used in the treatment of lymphomas. The possibility exists that, in combination therapies involving cytotoxic agents and a glucocorticoid, a steroid-resistant lymphoid cell variant induced by a drug might be selected by the presence of the hormone.

REFERENCES

M. Huet-Minkowski et al.


Induction of Glucocorticoid-resistant Variants in a Murine Thymoma Line by Antitumor Drugs

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