The Cell Cycle Related Differences in Susceptibility of HL-60 Cells to Apoptosis Induced by Various Antitumor Agents

Wojciech Gorczyca, Jianping Gong, Barbara Ardelt, Frank Traganos, and Zbigniew Darzynkiewicz

ABSTRACT

The studies were aimed to detect the cell cycle-associated differences in the susceptibility of HL-60 cells to apoptosis induced by diverse agents. Exponentially growing HL-60 cells were treated with the DNA topoisomerase I inhibitor camptothecin; the DNA topoisomerase II inhibitors teniposide, m-AMSA, Mitoxantrone, or Fostriecin; the presumed tyrosine kinase inhibitor genestein; a serine/threonine kinase inhibitor H7; the protein synthesis inhibitor cycloheximide; the DNA replication inhibitor hydroxyurea; the nucleotide antimetabolites 1-β-D-arabinofuranosylcytosine and 5-azacytidine; and the alkylating agent nitrogen mustard, cisplatin, hyperthermia, and γ irradiation. Endonucleolysis, which accompanied apoptosis induced by these agents, was assessed by two different flow cytometric methods, one based on DNA content measurements following extraction of low molecular weight DNA, and another using exogenous terminal deoxynucleotidyl transferase to label in situ DNA strand breaks. Each method allowed for both identification of apoptotic cells and analysis of the cell cycle distribution of the unaffected cell population; the method using terminal transferase also allowed for identification of the cell cycle position of apoptotic cells. Confirmed by analysis of DNA degradation by gel electrophoresis and changes in cell morphology, apoptosis was observed as early as 3 h after administration of most drugs and for some drugs was cell cycle phase specific. Cells progressing through S phase were selectively susceptible when treated with camptothecin, teniposide, m-AMSA, Mitoxantrone, H7, hydroxyurea, and 1-β-D-arabinofuranosylcytosine. Cells in G2-M preferentially underwent apoptosis in cultures treated with H7 or with γ-irradiation. Cells in G0, phase were preferentially affected by 5-azacytidine, nitrogen mustard, and hyperthermia. No significant cell cycle specificity was observed in the case of Fostriecin, genestein, cycloheximide, or cisplatin. The cell cycle related difference in susceptibility to apoptosis may be a reflection of both the severity of the lesion induced by a given drug and the ability of the cells to repair that lesion; both can vary depending on the cell cycle phase.

INTRODUCTION

There is a growing body of evidence that the efficacy of various antitumor agents is related to the intrinsic propensity of the target tumor cells to respond to these agents by apoptosis (for reviews, see Refs. 1–3). The apoptotic mode of cell death involves an active participation of the affected cell in its self-destruction via activation of a preprogrammed cascade of molecular events that culminate in DNA degradation, nuclear disintegration, and packaging of cell remnants into "apoptotic bodies," which are then rapidly removed by macrophages (for reviews, see Refs. 4–7).

The role of cell proliferation and cycle specific factors in modulating the apoptotic response has been the subject of several recent studies (8–15). Constitutive expression of c-myc, a prerequisite for the continuous proliferation of human fibroblasts, was shown to be an essential factor for these cells to respond to removal of growth factors by apoptosis (9–12). The high susceptibility of HL-60 or COLO 320 cells to undergo apoptosis in response to a variety of toxic agents also appears to be associated with overexpression of c-myc (12). Such cells may already be "primed" to respond by apoptosis via constitutive expression of an apoptosis-associated endonuclease (13). The role of cell cycle progression has been emphasized in studies of Colombel et al. (14), who observed an entrance into an "abortive" cell cycle of cells committed to apoptosis, prior to their death.

We have recently reported that apoptosis of HL-60 cells seen shortly (>2 h) after administration of DNA topoisomerase I and II inhibitors CAM, TN, or m-AMSA was preferential to S phase cells (15–18). No cell cycle specificity, however, was observed by Cotter et al. (19) in the case of T-cell hybridoma cells treated with CAM or aphidicolin for 16 h. It is unclear whether this discrepancy is due to differences in the cell lines used in the respective studies (HL-60 versus T-cell hybridoma) or to differences in the duration of drug treatment. Induction of apoptosis in other T-cell lines (MOLT-4 and L1210) was seen to differ markedly compared to that observed in promyelocytic HL-60 cells (15).

Several recently developed flow cytometric methods can identify apoptotic cells and simultaneously relate their position in the cell cycle (for a review, see Ref. 20). Two such techniques were used in the present study to provide more definite evidence regarding the sensitivity of HL-60 cells to a variety of antitumor drugs, hyperthermia and γ-irradiation. One method is based on DNA content analysis of ethanal fixed and permeabilized cells following extraction from these cells of the degraded, low MW DNA. Another method uses exogenous TDt to label in situ the DNA strand breaks with b-dUTP (18, 21, 22). As mentioned, the cell cycle specificity of apoptosis induced by CAM, TN, and m-AMSA has already been described by us (15–18). Additional, more extensive studies on these and several other drugs are presented in this report.

MATERIALS AND METHODS

Cell Culture. The HL-60 cells were maintained in RPMI 1640 (GIBCO BRL Life Technologies, Inc., Grand Island, NY) supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mm L-glutamine. The cells were split every third day and were diluted 1:2 1 day before each experiment. Cell densities in cultures did not exceed 5 × 10⁴ cells/ml.

Drugs. CAM (Sigma Chemical Co., St. Louis, MO) was diluted in DMSO at a concentration of 1 mM and stored at −20°C. TN was kindly provided by Henry H. Holava of Bristol Myers Co. (Wallingford, CT). A stock solution (50 mM) of TN was prepared in DMSO and stored at −20°C. FST (Ben Venue Laboratories, Inc., Bedford, OH), was kindly provided by Dr. Werbel of Parke-Davis (Ann Arbor, MI). FST stock solutions (5 mM) were prepared by adding 2 ml of Hanks' buffered salt solution to the lyophilized substance with further

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3 The abbreviations used are: DMSO, dimethyl sulfoxide; b-dUTP, biotinylated dUTP; TdT, terminal deoxynucleotidyl transferase assay; AZC, 5-azacytidine; CAM, camptothecin; CHX, cycloheximide; FST, Fostriecin, phosphotriene; GEN, genestein; HKU, hydroxyurea; m-AMSA, amarsamine; 4'-[9-acridynylamino]-3-methanesulfonyl-m-anisidide; MIT, Mitoxantrone (Novantrone); TN, teniposide, VM-26; MW, molecular weight; PBS, phosphate buffered saline.
dilutions made in RPMI 1640. Both CHX (Sigma) and MIT (American Cyanamid, Pearl River, NY) were prepared as stock solutions at a concentration of 5 mM in distilled water prior to each experiment. Stock solutions of the protein kinase C inhibitor H7 (Seikagaku America, Inc., Rockville, MD) were prepared at a concentration of 10 mM in distilled water and stored at -20°C. Nitrogen mustard was obtained from Sigma and the stock solution was prepared in distilled water at a concentration of 1 mM. All solutions were prepared freshly prior to experiments. Cell irradiation was carried out at the dose rate of 10 Gy/min using a cesium-137 irradiator (Campagnie Oris Industrie; Model IBL-437C), as described (17). Hyperthermia was induced by heating cells in a water bath at 43°C for 30 min. The cells were treated with the respective drugs for 3 to 6 h, as indicated in the Table 1; the drug concentrations are also presented elsewhere (18, 21). It should be noted that discrimination of apoptotic cells in this assay depends on the proportion of DNA extracted from these cells prior to the measurement, which can be modulated by the variation in concentration of ethanol used for fixation (within a range between 50 and 80%; a higher proportion of DNA is extracted after cell fixation at lower ethanol concentrations) and the duration of the extraction (10-60 min).

**In Situ TdT Assay.** Aliquots of cells were removed from control and drug treated HL-60 cultures, concentrated by centrifugation at 900 rpm for 5 min, and fixed in 1% buffered formaldehyde (pH 7.4) for 15 min on ice. After washing in PBS, cells were resuspended in 70% cold (~20°C) ethanol and transferred to the freezer where they were stored for up to 3 days. After rehydrating in PBS, the cells were resuspended in 50 μl of a caddyculate buffer containing 0.2 M potassium cacodylate; 2.5 mM Tris-HCl (pH 6.6); 2.5 mM CoCl2; 0.25 mg/ml bovine serum albumin; 5 units of terminal deoxynucleotidyl transferase; and 0.5 mmol b-dUTP (all reagents were purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN). The cells were incubated in this solution at 37°C for 30 min; rinsed in PBS; resuspended in 100 μl of a solution containing 4X concentrated saline-sodium citrate buffer (Sigma), 2.5 μg/ml fluoresceinated avidin (Boehringer), 0.1% Triton X-100, and 5% (w/v) non-fat dry milk; incubated in this solution for 30 min at room temperature in the dark and then rinsed in PBS containing 0.1% Triton X-100; and resuspended in 1 ml of PBS containing 5 μg/ml of propidium iodide (Sigma) and 0.1% RNase (Sigma). Control samples were treated identically except that the incubation medium was lacking the terminal transferase enzyme. Green (b-dUTP) and red (DNA) fluorescence of individual cells was measured on a FACScan Flow Cytometer (Becton Dickinson, San Jose, CA). The data from 10⁴ cells/sample were collected and stored using Lysys II software. Further details were presented before (15-18, 21, 22).

**DNA Gel Electrophoresis.** Untreated or drug treated HL-60 cells were collected by centrifugation, washed in PBS (without Ca²⁺ and Mg²⁺), resuspended (5 x 10⁶ cells) in 0.5 ml of 45 mM Tris-borate buffer-1 mM EDTA, pH 8.0, containing 0.25% Nonidet P-40 (Sigma) and 0.1% RNase A (Sigma), incubated at 37°C for 30 min and then treated with 1 mg/ml of proteinase K (Boehringer), and incubated for an additional 30 min at 37°C. After incubation, 0.1 ml of loading buffer (0.25% bromophenol blue-0.25% xylene cyanol FF-30% glycerol) was added and 25 μl of the tube content were transferred to a 1% agarose gel, transferred to nitrocellulose and processed as described (23).

**Table 1** Effect of different drugs, hyperthermia, and γ-irradiation, at concentrations and doses which rapidly induce apoptosis of HL-60 cells, on cell cycle distribution of the nonapoptotic cell population

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n°</th>
<th>G1</th>
<th>S</th>
<th>G2 + M</th>
<th>Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>39 ± 3</td>
<td>47 ± 5</td>
<td>14 ± 3</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>CAM (0.15 μM; 3 h)</td>
<td>8</td>
<td>40 ± 6</td>
<td>45 ± 5</td>
<td>15 ± 5</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>CAM (1 μM; 3 h)</td>
<td>5</td>
<td>77 ± 6</td>
<td>10 ± 5</td>
<td>13 ± 2</td>
<td>31 ± 9</td>
</tr>
<tr>
<td>TN (1 μM; 3 h)</td>
<td>5</td>
<td>74 ± 2</td>
<td>10 ± 3</td>
<td>16 ± 3</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>m-AMSA (1 μM; 6 h)</td>
<td>2</td>
<td>66 ± 5</td>
<td>22 ± 7</td>
<td>11 ± 5</td>
<td>31 ± 8</td>
</tr>
<tr>
<td>m-AMSA (1 μM; 6 h)</td>
<td>2</td>
<td>76 ± 4</td>
<td>8 ± 5</td>
<td>16 ± 1</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>m-AMSA (2 μM; 6 h)</td>
<td>3</td>
<td>58 ± 3</td>
<td>12 ± 1</td>
<td>30 ± 3</td>
<td>48 ± 11</td>
</tr>
<tr>
<td>MIT (1 μM; 4 h)</td>
<td>2</td>
<td>62 ± 9</td>
<td>10 ± 8</td>
<td>28 ± 5</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>BST (50 μM; 4 h)</td>
<td>3</td>
<td>63 ± 2</td>
<td>22 ± 6</td>
<td>14 ± 6</td>
<td>31 ± 8</td>
</tr>
<tr>
<td>H7 (50 μM; 6 h)</td>
<td>30</td>
<td>44 ± 3</td>
<td>38 ± 1</td>
<td>18 ± 6</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>GEN (0.2 mM; 6 h)</td>
<td>2</td>
<td>71 ± 9</td>
<td>20 ± 5</td>
<td>9 ± 4</td>
<td>36 ± 18</td>
</tr>
<tr>
<td>1-β-arabinofuranosylcytosine (25 μM; 4 h)</td>
<td>2</td>
<td>32 ± 5</td>
<td>48 ± 5</td>
<td>20 ± 2</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>HXU (0.65 mM; 4 h)</td>
<td>2</td>
<td>80 ± 5</td>
<td>8 ± 3</td>
<td>11 ± 1</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>AZC (0.4 mM; 4 h)</td>
<td>2</td>
<td>76 ± 8</td>
<td>15 ± 5</td>
<td>8 ± 2</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>Nitrogen mustard (2 μM; 6 h)</td>
<td>3</td>
<td>30 ± 4</td>
<td>17</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Cisplatin (50 μM; 4 h)</td>
<td>3</td>
<td>31 ± 14</td>
<td>52 ± 16</td>
<td>17 ± 1</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>CHX (20 μM; 3 h)</td>
<td>2</td>
<td>41 ± 9</td>
<td>17 ± 9</td>
<td>30 ± 26</td>
<td></td>
</tr>
<tr>
<td>Hyperthermia (43°C; 30 min, 6 h)</td>
<td>2</td>
<td>42 ± 5</td>
<td>52 ± 6</td>
<td>6 ± 1</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>Radiation (25 Gy; 3 h)</td>
<td>3</td>
<td>56 ± 11</td>
<td>35 ± 10</td>
<td>9 ± 1</td>
<td>39 ± 3</td>
</tr>
</tbody>
</table>

* n°, number of experiments.
* Percentage of cells in different phases of the cycle, and percentage of apoptotic cells, was estimated based on DNA staining with diamidino-2-phenylindole or diamidino-2-phenylindole and sulforhodamine, as shown in Figs. 1 and 2.
* TdT assays (see Fig. 3).
* 2 μM AZC.
* Hyperthermia 43°C for 30 min followed by 6 h incubation at 37°C.
the gel. Horizontal 1.5% agarose gel electrophoresis was performed at 2 V/cm for 6 h and the DNA in gels was visualized under UV light after staining with ethidium bromide (5 μg/ml; Polysciences, Inc., Warrington, PA).

RESULTS

Exposure of exponentially growing HL-60 cells to each of the drugs, to hyperthermia or γ-radiation, led to apoptosis of a large cell subpopulation (Figs. 1-3; Table 1). The effect was rapid and, within 3–6 h of the treatment, flow cytometric measurement of DNA content demonstrated the presence of cells with a fractional DNA content, typical of apoptosis (20).

The cell cycle analysis of the unaffected cell population in the treated cultures revealed marked differences in the proportion of cells in the respective phases of the cycle. Thus, a pronounced loss of S phase cells, from 47 to 10, 12, 22, 20, 8, and 15% was evident in cultures treated with CAM, TN, m-AMSA, MIT, H7, 1-β-D-arabinofuranosylcytosine, and HXU, respectively. No significant changes in the proportion of S phase cells were observed in cultures treated with FST, GEN, cisplatin, or CHX, while an increase in the percentage of cells in S was observed in cultures exposed to AZC and hyperthermia.

Changes in the proportion of cells in G2-M were also seen in the case of some drugs. Thus, whereas a loss of G2-M cells, from 14 to 6, 8, or 9% was encountered in the irradiated (25 Gy), HXU, or H7-treated cultures, the increase in proportion of these cells to 30, 22, and 20% was apparent as a result of the treatment with m-AMSA, FST, and GEN, respectively. The deficit in the proportion of unaffected cells in G1 phase, from 39 to 24, 31, and 31%, coinciding with the

Fig. 1. Frequency distribution DNA histograms of HL-60 cells, control cells, and cells treated with different drugs and γ-irradiation. Treatment induced appearance of cells with fractional DNA content (Ap) coincides with changes in the cell cycle distribution of the remaining cells. Note the loss of S phase cells from cultures treated with CAM, TN, m-AMSA, and H7 and an increase in the proportion of G2 cells in cultures treated with FST, m-AMSA, and GEN.
**CELL CYCLE RELATED DIFFERENCES IN SUSCEPTIBILITY TO APOPTOSIS**

**Protein**

![Image](image-url)

**DISCUSSION**

Two different flow cytometric methods were used in the present study to assay apoptosis, each allowing to estimate position of cells in the cell cycle. The first method was based on measurement of cellular DNA content. In this approach, the partially degraded DNA, a result of activation of an endonuclease in apoptotic cells (6, 7), was extracted from the ethanol fixed cells prior to their measurement and apoptotic cells were identified on the DNA frequency histograms (Fig. 1) or bivariate DNA/protein contour maps (Fig. 2) as the cells with a fractional DNA content (18). By comparing the cell cycle distributions of the unaffected cells (i.e., the cells with full DNA content) from the drug treated cultures with those from the control cultures, it was possible to estimate the cell cycle selectivity in the transition from the live to the apoptotic cell compartment. The disadvantage of this method is that when DNA degradation, in a given cell, is not much advanced, most DNA in that cell is still of high MW and, thus, is not extracted prior to measurement. This causes an overlap between the live and apoptotic cell populations which leads to underestimation of the latter. Such overlap was especially evident in samples treated with radiation at the internucleosomal, linker DNA sections. Fig. 4, representing cells treated with some of the drugs, illustrates the results that were typical for all the drugs presently tested, as well as following exposure to hyperthermia and γ-irradiation.
Fig. 3. Detection of secondary DNA strand breaks, associated with apoptosis, in cells treated with different drugs and \( \gamma \)-radiation. DNA strand breaks in apoptotic cells (Ap) are labeled with biotinylated dUTP in the reaction catalyzed by exogenous terminal deoxynucleotidyl transferase (18, 21); simultaneous counterstaining of DNA allows correlation of the presence of DNA strand breaks with cell position in the cell cycle. By gating analysis of the dUTP unlabeled and labeled cell populations, the cell cycle distribution of nonapoptotic and apoptotic cells, respectively, can be estimated (Table 1). In contrast to the staining procedure shown in Figs. 1 and 2, only a minor amount of DNA is extracted from apoptotic cells in this method. Note the S phase specificity of apoptosis after treatment with CAM, TN, or m-AMSA, but not after FST, G1 apoptosis following AZC, and G2 following \( \gamma \)-irradiation. Inset, DNA content frequency histograms of the total cell population (left), nonapoptotic (middle), and apoptotic (right) cell populations; the frequency scales are arbitrary (scaled to maximum frequency). Dashes, the gating thresholds.

MIT (data not shown); apparently the endonuclease induced by MIT was less extensive, compared to other DNA topoisomerase II inhibitors.

In the second assay, formaldehyde fixed and permeabilized cells were incubated in the presence of exogenous TdT and b-dUTP (18, 21, 22). The 3'-OH termini in DNA strand breaks served as primers for incorporation of b-dUTP. DNA strand breaks are labeled with b-dUTP under these conditions, and apoptotic cells, which contain a large number of such lesions, can be discriminated based on intense labeling. In contrast, fewer primary DNA strand breaks are observed in the irradiated cells or in cells treated with topoisomerase inhibitors (in the "cleavable complexes"), and such cells are labeled to a much lesser degree compared to apoptotic cells (21, 22). Since, unlike in the first method, the cross-linking agent formaldehyde is used as a fixative, the extraction of DNA from apoptotic cells during the procedure is minor. The method is very sensitive and the DNA strand breaks are detected...
CELL CYCLE RELATED DIFFERENCES IN SUSCEPTIBILITY TO APOPTOSIS

very early during apoptosis, prior to changes in cell morphology (18, 21, 22). Additional evidence of drug induced apoptotic cell death was obtained by gel electrophoretic analysis of the DNA degradation products (Fig. 4) and by microscopy (data not shown, but see Refs. 21 and 22).

In the cultures treated with CAM, TN, m-AMSA, MIT, H7, 1-ß-D-arabinofuranosylcytosine, HXU, or radiation, the appearance of apoptotic cells with fractional DNA content (Figs. 1 and 2) coincided with the loss of cells in S or G2 phases of the cell cycle. We interpret these results as indicating that cells in these phases (or at least the S phase cells) were selectively susceptible to apoptosis induced by the above drugs. The rationale for this interpretation is as follows: (i) No significant change in total cell number was observed in these cultures in the course of the experiment; thus, the appearance of cells in one compartment (Ap) is expected to be compensated for by their loss from particular phase(s) of the cell cycle. (ii) The treatment duration was rather short (3 h for most drugs) compared with the cell cycle time of the HL-60 line (22 h); therefore, even if the cells were arrested by the drug in a particular phase of the cycle, while the progression through the remainder of the cycle was unperturbed, this time interval was adequate for marked changes in the cell cycle distribution, such as observed (e.g., Figs. 1, D-F and H, and 2, B and D), to occur. (iii) At lower doses most drugs used in this study slowed down the progression of HL-60 cells through the S and G2 phases of the cycle and at subapoptotic doses the progression was completely halted (e.g., Ref. 25). To explain cell loss from these phases (as presently seen) solely on the basis of a phase specific cell arrest, one has to assume that at higher drug concentrations, the cells actually traversed S and G2 at accelerated rates (i.e., much faster than in control) and that the block was in G1. This is very unlikely. (iv) The most convincing and direct evidence that some of the drugs preferentially induce apoptosis of cells at particular phases of the cycle was provided by the TdT assay; i.e., because the loss of DNA from apoptotic cells was minor in this assay, the cell cycle position of both the unaffected and apoptotic cells could be estimated. The raw data unequivocally demonstrate, for example, that apoptotic cells are S-phase cells in CAM, TN, and m-AMSA treated cultures, predominantly G1 cells in AZC treated culture, and G2-M cells in y-irradiated culture (Fig. 3).

Concomitant with the loss of cells in S phase, an increase in the proportion of G2 cells was seen in m-AMSA treated culture (Table 1). This may reflect the strong effect of this drug in arresting cells in G2 (14). Namely, initially during incubation with the drug, the cells were entering G2 and had become arrested therein; these cells, in contrast to the cells still progressing through S, were resistant to apoptosis. An increase in the proportion of cells in G2, although less pronounced, was also observed in the case of FST and GEN. This result is compatible with the known propensity of these drugs to arrest cells in G2 (26, 27), and, as presently seen, to induce apoptosis in all phases of the cycle. As in the case of FST and GEN, little cell cycle specificity in induction of apoptosis was demonstrated by CHX or cisplatin.

The high sensitivity of S phase cells to CAM, TN, m-AMSA, or MIT may be a reflection of the mechanism of cell death postulated by Hsiang et al. (28) and Holm et al. (29); collisions of the DNA replication forks with the "cleavable complexes," if frequent, may directly trigger apoptosis. Indeed apoptosis of S phase HL-60 cells induced by CAM can be prevented by aphidicolin, an inhibitor of DNA replication (18), whereas FST, a topoiso merase II inhibitor which does not stabilize "cleavable complexes," does not induce preferential apoptosis of S phase cells.

It is more difficult to characterize the nature of lesions induced by H7 or GEN that trigger apoptosis. GEN, a tyrosine kinase inhibitor (30), and H7, a serine/threonine kinase inhibitor (31), may both act indirectly; e.g., H7 inhibits protein kinase C phosphorylation of topoisom erases which, in turn, may be necessary to prevent apoptosis (32). Alternatively, a more direct interaction may occur since both GEN and H7 bind at or near the ATP binding domains of their respective kinases the structure of which is highly conserved and shows significant homology to the ATP binding domain of the topoisomerase II enzyme (33).

We have previously reported (15) that CAM, TN, and m-AMSA, at concentrations lower than those which trigger immediate (i.e., seen after 2–4 h) apoptosis of HL-60 cells, perturb cell progression through S (CAM) or S and G2 (TN; m-AMSA). Likewise, MIT, FST (27), GEN (26), H7 (34), CHX (18), and radiation (15), at lower doses all perturb the cycle progression of HL-60 cells. Generally, the phase of the cell cycle through which cell progression is perturbed by low concentration of a particular drug is the same at which higher drug concentrations induce apoptosis (35). This suggests the presence of a drug-induced damage assessment mechanism, which is associated both with the cell cycle progression regulatory mechanism and with the trigger of apoptosis. If the damage is recognized as repairable, the cell slows down its progression through the cycle and attempts to repair the damage. Conversely, apoptosis is triggered if the damage is extensive and perhaps irreparable. Some drugs, however, such as doxorubicin or MIT, have a broad range of concentrations which suppress cell cycle progression (14) and only at very high doses induce immediate apoptosis.

The present studies were focused on immediate apoptosis which occurs in HL-60 cells within 2–6 h after administration of the drug. As discussed, it is the primary lesions induced by the drugs which appear to be associated with triggering apoptosis that quickly. Mechanisms of delayed apoptosis, observed at lower drug concentrations and after longer time intervals (often equivalent to the cell cycle duration), may be different and involve secondary lesions, related to unbalanced growth, inefficiency of DNA repair, and other factors, e.g., such as those discussed by Kung et al. (36). The difference in time may also explain the discrepancy between our present data, showing the cell cycle specificity of some of the drugs versus the data of Cotter et al. (19), who studied apoptosis after a prolonged period of drug exposure.

It is difficult to assess, at present, to what extent "immediate" apoptosis, induced by relatively high, although still pharmacological, drug concentrations, plays a role in chemotherapy. The appearance of a large proportion (>70%) of apoptotic blast cells in the blood of leukemic patients as early as 8 h after administration of the DNA...
topoisomerase inhibitors MIT or etoposide (VM-16; Ref. 21) indicates, however, that this mode of cell death is of relevance in the clinic.

The cell cycle related difference in cell susceptibility to apoptosis which was presently observed in the case of some drugs, is, most probably, a reflection of both the severity of the lesion induced by a given drug versus the ability of the cells to repair that lesion. Both can vary depending on the cell cycle phase. Knowledge of the cell cycle factors modulating cell response by apoptosis may be helpful in designing treatment protocols. The optimal drug combinations applied to tumors classified as the same may be quite different if the cell cycle distributions of the tumor cell populations differ. As the present studies suggest, whereas CAM, TN, or m-AMSA may be more effective for tumors with high S phase fractions, FST, AZC, nitrogen mustard, or hyperthermia may be preferred to treat tumors with low fraction of proliferating cells. The latter drugs may also be more effective in the intervals following CAM, TN, or m-AMSA to eradicate the cells that did not enter S phase.

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