The Effect of Topoisomerase Inhibitors on the Expression of Differentiation Markers and Cell Cycle Progression in Human K-562 Leukemia Cells

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Treatment of human K-562-J leukemia cells for 1 h with the topoisomerase II-reactive drugs VP-16, VM-26, or mAMSA resulted in a dose-dependent inhibition of proliferation and in an increase in the percentage of cells staining positive for hemoglobin, a marker of erythroid differentiation. Staining for hemoglobin of up to about 60% of the cells was observed at 20 µM VP-16, 1 µM VM-26, and 8 µM mAMSA. Such treatment also caused a G2/M arrest in the cell cycle. Incubation of the cells with radiolabeled VP-16 indicated that the induced erythroid differentiation was not due to continuous cell exposure to a residual amount of the drug. VP-16-induced erythroid differentiation was also not affected by DNA, RNA, or protein synthesis inhibitors. Differentiation induction and the G2/M arrest evoked by VP-16, VM-26, and mAMSA were, however, reduced in the presence of novobiocin. Our results indicate that topo-reactive drugs that cause G2/M arrest in the K-562-J cell cycle can induce these cells erythroid differentiation after a short and irreversible interaction with their target molecule(s).

INTRODUCTION

Topoisomerase (topo)3 inhibitors are valuable tools for studying the biological roles of their target enzymes. The eukaryotic topoisomerase II inhibitors can be classified into two groups. The first includes agents such as the antibiotics novobiocin and coumermycin A1, which are more effective in inhibiting protaryotic than eukaryotic topo II [1–3]. The mode of action of these inhibitors seems to involve interference with the ATPase function of the enzyme [4, 5]. The second group encompasses a diversity of anticancer drugs, including VP-16, VM-26, and mAMSA. These drugs are collectively known as topo II-reactive drugs, or topo II poisons, and their inhibitory effect involves stabilization of the reaction intermediate between topo II and DNA [5, 6]. The topo I inhibitor camptothecin operates in a similar manner, stabilizing the reaction intermediate between topo I and DNA [7].

Topoisomerases have been suggested to play a role in DNA replication [8, 9], cell division [9, 10], and gene expression [11]. Topo II activity and content increase in proliferating cells [12–15] and decrease in differentiated cells [13–23]. Moreover, topo II inhibitors have been shown to induce differentiation in human and murine leukemia cells and in human melanoma cells [18–24]. Based on these results, we suggested that chemically evoked reduction in topo II activity may lead to differentiation in human leukemia and melanoma cells [18–20].

The present studies were initiated to determine whether a short treatment (1 h) with topo inhibitors such as VP-16, VM-26, mAMSA, or camptothecin is sufficient to initiate events that lead to erythroid differentiation in human K-562-J leukemia cells and whether these events require (i) the synthesis of DNA, RNA, or protein or (ii) changes in cell cycle progression.

MATERIALS AND METHODS

Chemicals and reagents. Novobiocin was obtained from Sigma Chemical Co., mAMSA and camptothecin from the National Cancer Institute, and VP-16 and VM-26 from Bristol-Myers, Wallingford, Connecticut. Stock solutions of novobiocin dissolved in double-distilled water; VP-16, VM-26, or camptothecin in dimethyl sulfoxide (DMSO); and mAMSA in ethanol were stored at 70°C. [3H]VP-16 (100 mCi/mmol) dissolved in ethanol was purchased from Moravek Biochemicals, Brea, California. Aphidicolin, cordycepin, and cycloheximide (Sigma) were dissolved in DMSO. [methyl-3H]Thymidine (56 Ci/mmol), [H]Juridine (8 Ci/mmol), and [14C]methionine (55 mCi/mmol) were purchased from New England Nuclear, Boston, Massachusetts. The source of topo I and topo II activities was K-562-J cell extracts [18].

0014-4827/92 $5.00
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Cells, culture conditions, drug treatment, and assessment of differentiation.

For our studies we used homogenous cell populations. The K-562-J cells, which can differentiate along the erythroid pathway, derived from a cell clone isolated by Dr. S. Maeda in this laboratory from the human K-562-J leukemia cell line [19]. SK- MEL-131 cells (clone 3.44) were obtained from Dr. Alan Houghten, Memorial Sloan-Kettering Cancer Center, New York, New York [25]. For enzyme assays and Western blotting, cells were incubated at 1.5 × 10^6 cells/ml of growth medium containing RPMI 1640 supplemented with 15% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) and cultured at 37°C in a humidified atmosphere of 8% CO2 in air. For differentiation studies (continuous drug treatments), cells were incubated at 5 × 10^6/ml and treated for the 5-day duration of the experiment with VP-16, VM-26, mAMSA, camptothecin, novobiocin, or N-methyl-N-nitroso-urea. For short treatments, cells incubated at 5 × 10^6/ml were treated for 1 h with the appropriate topo-reactive drug or without 1-h preincubation with novobiocin; novobiocin was also present during the 1-h treatment with the drug. For the short treatment, the cells were washed by centrifugation at 500g followed by resuspension of the cell pellet in 10 ml of fresh growth medium. This process of cell washing was repeated three more times, after which the cells were resuspended at the original cell concentration and incubated at 37°C in a humidified atmosphere for up to 6 days. The effect of cell washing on the removal of VP-16 was determined after treatment of a cell suspension containing 5 × 10^6/ml cells with 20 μM [3H]VP-16 (0.5 μCi). The radioactivity of the original culture and after each washing was determined by scintillation counting. Aliquots of the cultures were taken daily to establish the total number of cells and the number of viable cells. Cell viability was determined by the trypan blue dye exclusion method and by the 5-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [26, 27]. We have, however, chosen to present cell viability data based on the trypan blue dye exclusion method, because we have found it to be more sensitive than the MTT assay in assessing the viability of the individual cells. Other aliquots were taken to determine the percentage of cells that stained positively for hemoglobin by a benzidine staining assay [28] or that reacted positively with the myeloid maturation-specific monoclonal antibody OKM1 [18]. Cellular melanin content was determined by densitometric analysis of a cell suspension applied to a thin layer chromatogram (TLC) plate (E. Merck, Darmstadt, Germany) as previously described [29]. The SK-MEL-131 cells were collected by treating with phosphate-buffered trypsin saline and washing in growth medium, followed by two additional washes in phosphate-buffered saline (PBS). Cells were then suspended in PBS at 2.5 × 10^6 cells/ml, and 2 μl of the cell suspension was applied to a TLC plate preactivated by heating at 120°C for 1 h. Various amounts of synthetic DOPA-melanin were dissolved in PBS and applied to the TLC plate as calibration standards. The relative absorbance at 400 nm of the samples was quantified by densitometric scanning of the TLC plate in the reflectance mode. Peak areas were determined with a Shimadzu CRIR data processor.

Immunoblotting. Immunoblotting was performed as previously described [18]. The primary antibody, anti-topo II IgG was prepared against the carboxyl terminal portion of the human topo II from the expression vector p56ZII-1-8 provided by L. Liu. The secondary antibody was anti-rabbit IgG. Detection was via peroxidase–antiperoxidase (Sigma).

Flow cytometry analysis. Changes in cell cycle progression after treatment with topo-reactive agents (VP-16, VM-26, mAMSA, or camptothecin) preincubated with or without novobiocin were determined as previously described [30]. The cells were fixed at 1 × 10^6 cells/ml in 75% methanol and stained with 4′,6-diamidino-2-phenylindole in a 0.1% citrate solution. Flow cytometry patterns were obtained using a particle analyzing system (Partec AG, Basel, Switzerland) and were analyzed using a computer program from Phoenix Flow Systems, Inc. (San Diego, California).

FIG. 1. Erythroid differentiation (A) and proliferation inhibition (B) induced in K-562-J cells by topo-reactive drugs. Cells were treated continuously for 5 days with VP-16 (C), VM-26 ( ● ), mAMSA ( ● ● ), or camptothecin ( ● ). The results represent the mean of three different experiments each performed in duplicate. The data varied up to 20% of the mean. After 5 days, the control (DMSO-treated) culture contained 1.1 × 10^6 cells/ml with 4% of these being benzidine-positive.

RESULTS

Induction of differentiation by topo-reactive agents. The treatment of K-562-J cells for 5 days with the topo-reactive agents VP-16, VM-26, mAMSA, or camptothecin resulted in a dose-dependent inhibition of cell proliferation. VM-26 and camptothecin were, on a dose basis, the most effective, giving 80% inhibition of proliferation at 0.1 μM; treatment with 0.1 μM mAMSA or VP-16 caused 35% inhibition (Fig. 1). Treatment with the topo-reactive agents also caused a dose-dependent increase in the percentage of differentiated cells characterized by benzidine staining. This increase was followed, at higher drug concentrations, by a decline in the percentage of cells staining positive (Fig. 1). Maximal staining was reached at 1 μM VP-16, 0.05 μM VM-26, 0.5 μM mAMSA, and 0.1 μM camptothecin. At these concentrations, the drugs inhibited cell proliferation by up to 85%, with more than 80% of the cells being viable as determined by their ability to exclude trypan blue. Even though up to 60% of the cells stained positively with benzidine after treatment with the topo II inhibitors VP-16, VM-26, or mAMSA, a maximum of only
the degree of cell proliferation inhibition or cell viability and induction of benzidine staining by these agents.

To determine whether differentiation could be induced after a short treatment with topo-reactive agents (a condition that would be more amenable for mechanism studies), we treated the K-562-J cells for only 1 h with VP-16, VM-26, mAMSA, or camptothecin (Fig. 2). These inhibitors induced the erythroid cell differentiation marker at concentrations that were more than an order of magnitude higher than those optimal with continuous treatment. The most effective induction of this marker was obtained with 20 \( \mu \)M VP-16, 1 \( \mu \)M VM-26, 8 \( \mu \)M mAMSA, or 4 \( \mu \)M camptothecin (Fig. 2). VM-26 was also most effective for inhibiting proliferation; VP-16 was least effective. At these concentrations the drugs inhibited cell proliferation by up to 65%, with more than 90% of the cells being viable as determined by their ability to exclude trypan blue (Fig. 2). These topo II-reactive agents were also able to induce the K-562-J cells to exhibit reactivity with the OKM1 monoclonal antibody, which is indicative of myeloid cell differentiation (Table 1). This differentiation marker is also inducible by novobiocin in the parental K-562 cells [24].

To test the possibility that the differentiation after the 1-h treatments was due to a residual amount of the drug, we incubated the K-562-J cells with 20 \( \mu \)M radio-labeled VP-16, the dose most effective in inducing differentiation after 1 h of treatment (Fig. 2), and determined the amount of the drug left in the cells after cell washings. These washings eliminated all but 0.1% of the drug (Table 2). This amount of drug is more than an order of magnitude lower than the amount required to obtain optimal expression of the erythroid marker with continuous treatment.

**Effect of DNA, RNA, and protein inhibitors and novobiocin on differentiation induction by topo-reactive agents.** Treatment of K-562-J cells for 2 h with 3 \( \mu \)M

![Figure 2](image-url)  
**FIG. 2.** Erythroid differentiation (A) and proliferation inhibition (B) induced in K-562-J cells by topo-reactive drugs. The cells were treated for 1 h, and then washed three times and cultured for 5 days in fresh medium. Treatments were as follows: VP-16 (○), VM-26 (●), mAMSA (△), camptothecin (∇). The results represent the mean of two independent experiments, each performed in duplicate. The data varied up to 20% of mean. After 5 days, the control (DMSO-treated) culture contained 1.2 \( \times \) 10^6 cells/ml, with 2% of these being benzidine-positive.

30% of the cells stained following treatment with the topo I inhibitor camptothecin (Fig. 1).

On a dose basis, camptothecin was the most potent inhibitor of proliferation but the least efficient in inducing benzidine staining. Novobiocin, which belongs to another group of topo II inhibitors, caused at 150 \( \mu \)M a 90% reduction in K-562-J proliferation (with about 60% cell viability) but not a significant increase in the percentage of cells stained positively with benzidine. A similar situation was observed with the short-lived alkylating agent N-methyl-N-nitrosourea, which caused at 0.2 mM a 25% reduction in cell proliferation (with about 90% cell viability) and at 1 mM a 60% reduction in cell proliferation (with about 40% cell viability) without increasing substantially the percentage of cells stained positively with benzidine. Also, incubation of K-562-J cells for 1 h with 1 mM hemin (an effective inducer of erythroid differentiation in these cells after continuous treatment) caused a 40% reduction in cell proliferation (with about 90% cell viability) without increasing substantially the percentage of cells stained positively with benzidine. These results indicate a dissociation between

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**TABLE 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cells reacting with antibody OKM1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>20 ± 14</td>
</tr>
<tr>
<td>VP-16 (20 ( \mu )M)</td>
<td>63 ± 11</td>
</tr>
<tr>
<td>VM-26 (1 ( \mu )M)</td>
<td>67 ± 15</td>
</tr>
<tr>
<td>mAMSA (4 ( \mu )M)</td>
<td>78 ± 19</td>
</tr>
</tbody>
</table>

* Concentrations of inhibitors were chosen so that the cell viability remained above 90%.
* Reactivity with OKM1 monoclonal antibody was evaluated 4 days after removal of the inhibitor, as described under Materials and Methods. The percentages are the means ± SE from at least three independent experiments.
TABLE 2
The Effect of Cell Washing on Removal of [\textsuperscript{3}H]VP-16 from K-562-J Cells Treated for 1 h with 20 \textmu M of the Drug

<table>
<thead>
<tr>
<th>Number of washes</th>
<th>cpm-5 \times 10^6 cells</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16,200</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>700</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Note.* Radioactivity before any washing (0 washes) was determined after the cells were centrifuged and resuspended in fresh medium.

aphidicolin, 2 \textmu M cordycepin, or 40 \textmu M cycloheximide (concentrations which were not toxic to the cells) resulted in a 60–70% inhibition of DNA, RNA, and protein synthesis, respectively (data not shown), as determined by the incorporation of the appropriate radiolabeled precursors [31]. Aphidicolin, cordycepin, or cycloheximide at these concentrations did not markedly affect cell proliferation or expression of the erythroid differentiation marker when incubated for 1 h prior to and during 1-h treatment with 20 \textmu M VP-16 (Table 3). Nor was differentiation affected when the cells were treated with 10-fold higher concentrations of aphidicolin, cordycepin, or cycloheximide, which caused more than 90% inhibition of macromolecular synthesis during the 2-h treatment. These treatments caused up to about 40% inhibition of cell proliferation (data not shown).

Treatment of the K-562-J cells for 2 h with 1 mM novobiocin resulted in 80–90% inhibition of DNA, RNA, and protein synthesis. This treatment did not affect the proliferation or differentiation of the K-562-J cells.

FIG. 3. The effect of novobiocin on erythroid differentiation (A) and proliferation (B) in K-562-J cells treated with VP-16. Cells were incubated for 1 h with (▲, △) or without (●, ○) 1 mM novobiocin prior to and then 1 h during treatment with the solvent (DMSO) (○, ●), or 20 \textmu M VP-16 (▲, △). After the treatment, the cells were washed three times, after which the cells were cultured for 5 days in fresh medium. The results represent the mean of two independent experiments, each performed in duplicate. The results varied up to 20% of the mean.

However, incubation of the cells with 1 mM novobiocin for 1 h prior to and then during 1-h treatment with 20 \textmu M VP-16 caused a more than a 50% reduction in the differentiation induced by VP-16 alone, without markedly affecting its inhibitory effect on proliferation (Table 3 and Fig. 3). Similar results were obtained after treatment with 1 \textmu M VM-26 or 4 \textmu M mAMSA (Fig. 4). Unlike these results, novobiocin had little or no effect on differentiation induction or growth inhibition evoked by 4 \textmu M camptothecin (Fig. 4).

To determine that the inhibitory effect of novobiocin on the expression of maturation markers induced by topo II-reactive drugs is not specific for K-562-J cells, we performed a similar experiment with SK-MEL-131 cells, which can be induced to express differentiation markers of mature melanocytes [20, 29]. Our results indicated that VP-16, VM-26, and mAMSA can induce in the SK-MEL-131 cells a twofold increase in melanin levels (Table 4). Novobiocin, which in itself did not affect the level of this melanocyte maturation marker, abrogated the increase in melanin levels induced by

TABLE 3
The Effect of Inhibitors on the Percentage of K-562-J Cells Stained Positively with Benzidine in Untreated (−) or VP-16-Treated (+) Cultures

<table>
<thead>
<tr>
<th>Inhibitor*</th>
<th>Cells stained positively with benzidine(^a) (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4 ± 1</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>Aphidicolin (3 \textmu M)</td>
<td>10 ± 5</td>
<td>51 ± 5</td>
</tr>
<tr>
<td>Cordycepin (2 \textmu M)</td>
<td>9 ± 3</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>Cycloheximide (40 \textmu M)</td>
<td>10 ± 2</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>Novobiocin (1 mM)</td>
<td>3 ± 1</td>
<td>12 ± 7</td>
</tr>
</tbody>
</table>

*Cells were incubated with aphidicolin, cordycepin, cycloheximide, or novobiocin 1 h prior to and 1 h during treatment with 20 \textmu M VP-16. After treatment, the cells were washed three times and incubated for 5 days.

\(^a\)The mean ± SE was determined from four independent experiments.
FIG. 4. The effect of novobiocin on erythroid differentiation and proliferation of K-562-J cells induced by topo-reactive drugs. Treatments with 20 μM VP-16, 1 μM VM-26, 4 μM mAMSA, 4 μM camptothecin, or 1 mM novobiocin were performed as described in the legend to Fig. 3. The results represent the mean ± SE of three independent experiments.

VM-26 and mAMSA and reduced the degree of the increase induced by VP-16 (Table 4).

Treatment of the K-562-J cells with 20 μM VP-16, 1

TABLE 4

<table>
<thead>
<tr>
<th>Drug</th>
<th>Melanin content* (μg/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>VP-16 (20 μM)</td>
<td>14.0 ± 2.1</td>
</tr>
<tr>
<td>VM-26 (1 μM)</td>
<td>14.2 ± 1.8</td>
</tr>
<tr>
<td>mAMSA (4 μM)</td>
<td>12.6 ± 3.0</td>
</tr>
</tbody>
</table>

* Novobiocin at a final concentration of 1 mM was incubated 1 h prior to and 1 h during treatment with the topo II-reactive drugs. After the treatment the cells were washed and incubated with fresh culture medium for 6 days after which time the level of melanin was determined.

† Concentrations of drugs were chosen so that the cell viability remained above 90%.

‡ The mean ± SE was determined from at least three independent experiments.

μM VM-26, 4 μM mAMSA, or 4 μM camptothecin for 1 h or 1 mM novobiocin for 2 h did not affect the cellular levels of topo II, as determined by immunoblotting with anti-topo II antibody of cellular extracts (Fig. 5). Nor were there detectable changes in topo II and topo I activities in K-562-J cell extracts prepared just after treatment, as measured by the unknotting and relaxation assays, respectively (data not shown).

FIG. 5. Immunoblotting of K-562-J cell extracts with anti-topo II antibody. Cells were treated with the topo inhibitors for 1 h (except novobiocin, for 2 h); lane 1, control; lane 2, 20 μM VP-16; lane 3, 1 μM VM-26; lane 4, 4 μM mAMSA; lane 5, 1 mM novobiocin.

Effect of novobiocin on G2/M arrest of the cell cycle induced by topo-reactive agents. The treatment of K-562-J cells for 1 h with 20 μM VP-16 caused a G2/M arrest in the cell cycle that reached its peak 20–30 h after initiating the treatment. During this period, about 85% of the cells accumulated in the G2/M phase. Similar results were obtained with 1 μM VM-26 or 4 μM mAMSA. Treatment of the K-562-J cells with 4 μM camptothecin also resulted in G2/M arrest, but to a lesser extent (Fig. 6). These G2/M accumulations returned to almost normal levels at 45 h after initiating the treatments with the drugs (data not shown).

Incubation of the K-562-J cells with 1 mM novobiocin 1 h prior to and during 1-h treatment with VP-16, VM-26, or mAMSA diminished the accumulation of cells at
FIG. 6.  G2/M arrest in the K-562-J cell cycle induced by topo
reactive drugs. The flow cytometric patterns were determined 20 h
after a 1-h preincubation without (A–E) and with (F–J) 1 mM novo-
biocin for 1 h. Treatments were as follows: A and F, DMSO solvent
(control); B and G, 20 μM VP-16; C and H, 1 μM VM-26; D and I, 4
μM mAMSA; E and J, 4 μM campto. DNA content is expressed in
relative units.

the G2/M phase induced by these topo II-reactive
agents, yet novobiocin had only little effect on the cell
cycle delay induced by campto (Fig. 6).

DISCUSSION

In this study we have shown that topo II-reactive
drugs such as VP-16, VM-26, or mAMSA can, in a dose-
dependent manner, induce in the K-562-J leukemia
cells the expression of an erythroid differentiation
marker: namely, staining with benzidine, which indi-
cates the presence of hemoglobin [28]. This differentia-
tion was detected in up to 60% of the cells. A similar
percentage of cells were induced by these drugs to ex-
press a myeloid maturation marker detected by reactiv-
ity with the OKM1 antibody. Thus, a fraction of the
treated K-562-J cells can express a phenotype charac-
teristic of both the erythroid and myeloid cell lineages.
However, the expression of these two maturation
markers in the K-562-J cells is not always coordi-
nated; novobiocin, an inhibitor of topo II that is not one of
the topo II-reactive drugs [1–3] induces in these cells the
myeloid [24], but not the erythroid, marker.

Expression of the erythroid differentiation marker
evoked by the topo-reactive drugs VP-16, VM-26,
mAMSA, or campto. was achieved not only by a
continuous 5-day treatment of the cells but also by a
short 1-h treatment. The acquisition of benzidine stai-
ning or reactivity with the OKM1 antibody in the K-562-
J cells after the 1-h treatment with VP-16, VM-26, or
mAMSA was associated with a G2/M arrest in the cell
cycle. Although this short treatment requires higher
doses of the inducer drugs, the expression of the dif-
ferentiation markers was most likely not due to residual
amounts of the drugs left after the cell washings, be-
cause, in the case of VP-16, (even if we assume that
there is no leaching of the drug from the cell into the
fresh medium), the amount of the drug remaining in the
cells was more than an order of magnitude lower than
the amount required to achieve erythroid differen-
tiation induction by continuous treatment. In this context,
it is interesting to note that studies with other related
leukemia cells indicated that by 90 min about 90% of the
drug is leaching into the fresh medium [32]. Thus, a 1-h
treatment with VP-16 is sufficient to commit K-562-J
cells to either an erythroid or a myeloid cell differen-
tiation.

Novobiocin induces myeloid differentiation in the hu-
mn HL-60 leukemia cells [18], murine WEHI –3b D+
leukemia cells [23], and human K-562 cells [24]. Yet
novobiocin was ineffective in inducing erythroid differen-
tiation or G2/M arrest in the K-562-J cells. In the
HL-60 cells, the induced differentiation was apparently
due to a reduced topo II activity resulting from proteo-
lytic degradation of the enzyme [18]. No such degrad-
ation or reduced topo II activity was evident in the novo-
biocin-treated K-562-J cells. This suggests that dif-
ferent mechanisms may control myeloid and erythroid
maturation processes induced by topo inhibitors in dif-
ferent leukemia cells.

Even though the ability of topo inhibitors to induce
differentiation is becoming established [16, 18–21, 23,
24], the underlying mechanism is still unknown. Our
results indicate that the erythroid differentiation
evoked by VP-16 was not affected by inhibitors of DNA, RNA, or protein synthesis but was markedly inhibited by novobiocin. The effect of novobiocin was not restricted to differentiation induction in the K-562-J cells, because novobiocin inhibited differentiation induction by topo II-reactive drugs in a human melanoma cell line as well. Novobiocin also abrogated the VP-16-evoked G2/M arrest in the K-562-J cell cycle, a feature of topo II-reactive drugs [33-37]. Similar effects were also observed in the case of VM-26 and mAMSA. These results reinforce the association between induction of differentiation and the G2/M arrest brought about by these drugs.

The mechanism by which novobiocin inhibits differentiation induction is not clear. One possibility is that novobiocin interacts with a protein different from topo II which is critical for both differentiation induction and cell cycle progression. In this context, it is interesting to note that VP-16 was recently shown to induce G2 arrest in association with the inactivation of p34cdc2 kinase, a cell cycle-controlling enzyme [34, 35]. It is thus possible that VP-16, VM-26, and mAMSA, either directly or after interaction with DNA and topo II, cause the inactivation of the p34cdc2 kinase or a related enzyme, which results in G2/M cell cycle arrest and differentiation induction in K-562-J cells. Such a mechanism would not necessarily require an inhibition of DNA, RNA, or protein synthesis. The mode of action of novobiocin may thus involve inhibiting the interaction between the topo II-reactive agents and this cell cycle-controlling enzyme.

Irrespective of these speculations, topo-reactive drugs which cause G2/M arrest in the cell cycle of K-562-J cells can induce in these cells erythroid differentiation after a short and irreversible interaction with a target molecule(s). DNA, RNA, or protein synthesis is most likely not required for this interaction.

The authors acknowledge the expert technical assistance of Ms. Jane Perrin in performing the flow cytometric analysis. This work was supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38 and NIH Grant CA-37435.

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Received January 22, 1992
Revised version received June 19, 1992