Novobiocin- and Phorbol-12-myristate-13-acetate-induced Differentiation of Human Leukemia Cells Associated with a Reduction in Topoisomerase II Activity

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ABSTRACT

Studies were conducted to determine the possible involvement of DNA topoisomerase II (Topo II) in the induction of differentiation in two human promyelocytic HL-60 leukemia cell variants that are either susceptible or resistant to differentiation induced by phorbol-12-myristate-13-acetate (PMA), a protein kinase C activator. The acquisition of maturation markers and changes in the activity, level, and phosphorylation of Topo II were determined after treatment with either novobiocin, a Topo II inhibitor, or PMA. Novobiocin at 50–150 μM induced differentiation in the HL-205 cells but not in the HL-525 cells, although both cell types were equally susceptible to novobiocin-evoked cytotoxicity. In both cell types, novobiocin induced similar reductions in topoisomerase I activity but different reductions in Topo II activity. Treatment with novobiocin at 150 μM for 6 h or at 2 mM for 30 min resulted in a 4-fold or higher reduction in Topo II activity in the differentiation-resistant HL-205 cells but not in the differentiation-resistant HL-525 cells. A differential response in Topo II activity was also observed after treatment with PMA. The novobiocin-evoked decrease in Topo II activity seems to be due to an enhanced enzyme proteinolysis, whereas the PMA-elicted decrease in Topo II activity is associated with an increase in Topo II phosphorylation.

INTRODUCTION

Topoisomerases are ubiquitous enzymes that change the conformation of DNA molecules. Their mode of action involves a transient breakage of one DNA strand (type I topoisomerase) or two DNA strands (type II topoisomerase) followed by rotation and rejoining of the strands (1, 2). In eukaryotes, these enzymes have been implicated in the replication and processing of DNA, as well as in the transcription of specific genes (3–9). Studies with mammalian cells have shown that the activity of Topo II is higher in replicating cells than in cells exhibiting a mature phenotype (10–16), thus suggesting that a reduction in Topo II activity may be associated with either inhibition of growth or the induction or maintenance of the differentiated state.

The present studies were initiated to distinguish between these possibilities and to determine specifically if a reduction in Topo II activity is a prerequisite for the induction of cell differentiation in the human promyelocytic leukemia HL-60 cells (17). In one approach, we tested the ability of novobiocin and related agents that inhibit Topo II activity (1, 2, 18–21) to induce differentiation in HL-60 cells. For comparison, we included camptothecin, an inhibitor of Topo I (22). In another approach, we tested the ability of PMA, a potent differentiation inducer (23–25), to reduce the activity of Topo II in the HL-60 cells shortly (within 30 min) after treatment. Because the biological activity of PMA is believed to involve specific protein phosphorylation induced by PKC (26), we examined the alterations in the activity, level, and phosphorylation of Topo II after treatment of the cells with either novobiocin or PMA in the presence and absence of H-7, an inhibitor of protein kinases, including PKC (27). Induction of cell maturation and changes in Topo II level, activity, and phosphorylation were tested in cell-line HL-205, a differentiation-susceptible HL-60 cell variant, and in cell-line HL-525, an HL-60 cell variant that is resistant to induction of differentiation by PMA but not by other types of inducers (28).

Our results indicate that novobiocin caused the HL-205 cells but not the HL-525 cells to acquire a mature myeloid phenotype. Furthermore, in the HL-205 cells but not the HL-525 cells, novobiocin and PMA were able to reduce the activity of Topo II and to alter the level of phosphorylated Topo II within 30 min after treatment. All of these changes were diminished by pretreatment of the cells with H-7. On the basis of these results, we suggest that induction of differentiation in HL-60 cells by some chemical agents is associated with a reduction in Topo II activity, which is facilitated by a protein kinase(s), perhaps PKC.

MATERIALS AND METHODS

Chemicals and Reagents. m-AMSA was obtained from the National Cancer Institute, and novobiocin from Boehringer Mannheim. Coumermycin was purchased from Sigma Chemical Co., PMA from Chemicals for Cancer Research; and H-7 from Seikagaku America, Inc. These chemicals, stored as stock solutions at −20°C, were used as follows. Novobiocin at 10 mM in sterile DDW, m-AMSA at 2.5 mM in 50% dimethyl sulfoxide in DDW, PMA at 1.6 mM in dimethyl sulfoxide, and H-7 at 10 mM in DDW. When a combination of H-7 and another drug was used, H-7 was added 1 h prior to the second drug. Bacteriophage P4 Vir1 del10 was provided by R. Calendar (University of California, Berkeley, CA). The MoP-9 monoclonal antibody was provided by A. Dimitriou-Bona (Mt. Sinai School of Medicine, New York, NY), the B52.1 monoclonal antibody was provided by G. Trinchieri (Wistar Institute of Anatomy and Biology, Philadelphia, PA), and two different rabbit IgG antibodies against mouse cell Topo II were provided by F. Drake (Smith Kline Beckman Co.). All other antibodies were purchased from either Ortho Pharmaceutical Corp., Coulter Immunology, or Becton Dickinson. A Topo II control sample was the active fraction of the Mono Q column partially purified from HL-60 cells as described by Drake et al. (29). Protein kinase C from rat brain was purified in our laboratory by J. Hardwick.

Cells, Culture Conditions, and Differentiation Markers. The HL-205 cell variant was isolated from HL-60 cells, while the HL-525 cell variant was obtained after cloning HL-60 cells that had been subcultured 102 times in the presence of PMA (28). Both cell variants were stable for...
at least 50–60 subcultures with regard to their susceptibility or resistance to PMA-induced cell differentiation. Cells were inoculated into 100-mm or 150-mm tissue culture dishes at 1.5 x 10^6 cells/ml of RPMI 1640 supplemented with 20% 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.

Immunofluorescence tests for reactivity with the antibodies, staining for NSE activity (30), and the NBT reduction assay (17) were all performed as previously described.

Preparation of Nuclear Extracts. Exponentially growing HL-205 or HL-525 cells in 150-mm culture dishes were treated with either the appropriate drugs or solvents only (controls). Following treatment, the cells were pelleted, lysed, and immunoprecipitated as described by Curran and Teich (35), except that the lysis buffer also contained 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 50 μg/ml leupeptin, 1 μg/ml pepstatin, and 20 μg/ml aprotinin. These nuclear extracts contained more than 90% of the total Topo II levels as determined by immunoblotting with the anti-Topo II antibodies (16, 18). The protein content of the nuclear extracts was determined by the Bradford method (31). Glycerol was added to a final concentration of 30%, and the extracts were stored at -20°C. These preparations served as the source for Topo I and II activity, as well as for Topo II immunoblotting.

Relaxation Assay for the Determination of Topoisomerase I Activity. The substrate used in the assay was supercoiled plasmid DNA isolated from the tailless capsids of the bacteriophage P4 Vir del as described by Liu et al. (32). Reaction mixtures of 20 μl contained 50 mM Tris-Cl, pH 8.0, 100 mM KCl, 10 mM MgCl2, 0.5 mM dithiothreitol, 0.5 mM EDTA, 30 μg/ml bovine serum albumin (nuclease free), and 1 mM ATP. Serial dilutions of the nuclear extracts served as the enzyme source. The reactions were started by the addition of 0.6 μg knotted DNA and terminated by the addition of 5 μl of a stop solution containing 5% SDS, 50 mM EDTA, 25% Ficoll, and 0.05 mg/ml bromophenol blue. Samples were loaded on 0.8% agarose gels. Electrophoresis was at 1.5 V/cm for 15 h in Tris-borate-EDTA buffer. Gels were stained in 1 mg/ml ethidium bromide, destained, and photographed under a UV light source. Densitometric scanning of the photographic negatives allowed the quantitative determination of the two DNA forms. One unit of unknotting activity was defined as the amount of enzyme that converts 50% of the substrate (knotted DNA) into the reaction product (relaxed DNA). The Topo II-DNA unknotting activity in the nuclear extracts was blocked by the omission of the enzyme cofactor ATP or by the addition of 0.5 mM novobiocin (Topo II inhibitor) but not by the addition of 1 mM camptothecin (Topo I inhibitor). Under these conditions, the Topo II activity is free from interference by Topo I activity and agents that may promote DNA degradation (33).

Immunoblotting and Immunoprecipitation of Phosphorylated Topoisomerase II. Immunoblotting was performed by the method of Towbin et al. (34) with a rabbit anti-Topo II IgG used as the primary antibody. The secondary antibody was peroxidase-conjugated anti-rabbit IgG (purchased from Sigma). Prior to their use, the antibodies were diluted 1:1000. For each preparation, 2 x 10^6 cells in their exponential growth phase were washed twice in serum-free, phosphate-free RPMI 1640 and then resuspended in 1 ml of this medium. After the addition of carrier-free [32P]Pi (0.2 μCi/ml), the cells were incubated for 2 h at 37°C, pelleted, and resuspended in serum-free RPMI 1640 containing phosphate and the appropriate inducer. After a 30-min incubation at 37°C, the cells were pelleted, lysed, and immunoprecipitated as described by Curran and Teich (35), except that the lysis buffer also contained 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 50 μg/ml leupeptin, 1 μg/ml pepstatin, and 20 μg/ml aprotinin. Following pelleting and washing, the protein A-Topo II complex was dissociated by boiling in 100 μl of Laemmli buffer (36) and electrophoresed on 8% SDSPolyacrylamide slab gels. The gels were stained, dried, and autoradiographed. Topo II phosphorylation was quantitated by densitometric determination of its M, 170,000 band.

RESULTS

Cell Differentiation Induction by Inhibitors of Topoisomerase II. Treatment of HL-205 cells for 7 days with up to 200 μM novobiocin, a Topo II inhibitor, resulted in a time- and dose-dependent cell growth inhibition (Fig. 1). This treatment also caused a fraction of the HL-205 cells to acquire a variety of myeloid maturation markers, which included reactivity with the OKM1, MoP-9 (Fig. 1), Mo2, My4, and B52.1 monoclonal antibodies (37–40). Other markers included NSE activity (41) and NBT reduction (17) (Table 1). However, this treatment did not cause the cells to attach to the surface of tissue culture plates as did PMA treatment (23, 24). The novobiocin-induced differentiation in the HL-205 cells was both time and dose dependent; e.g., a 7-day treatment with 50 μM novobiocin caused 25–40% of the cells to react positively with OKM1 and MoP-9 antibodies, while at 200 μM, the highest novobiocin concentration tested, more than 80% of the cells exhibited these markers (Fig. 1).

Treatments of the HL-525 cells (which are resistant to PMA-induced cell differentiation) with 50–200 μM novobiocin also caused cell growth inhibition. Furthermore, the degree of this effect was similar to that observed in the susceptible HL-205 cells after novobiocin treatment (Fig. 1). However, in the HL-525 cells, 50–150 μM novobiocin did not induce reactivity with either the OKM1, Mo2, My4, MoP-9, or B52.1 antibodies, nor did it cause an increase in NSE activity or an enhancement of NBT reduction (Fig. 1; Table 1), in contrast to the situation in the HL-205 cells. At 200 μM novobiocin, the most cytotoxic concentration tested, a modest increase in reactivity with the OKM1 antibody was observed (in less than 25% of the cells) (Fig. 1). However, novobiocin at this concentration did not induce any of the monocyte/macrophage markers.

HL-205 cells were also treated with the Topo II inhibitors coumermycin and m-AMSA, and the Topo I inhibitor camptothecin. On a dose basis, m-AMSA and camptothecin were about three orders of magnitude higher than novobiocin in cell growth inhibition and cytotoxicity induction while coumermycin was only about one order of magnitude higher. Of these, coumermycin and, to a lesser degree, m-AMSA, were also able to induce maturation markers in the HL-205 cells; however, the level of these markers did not reach that induced by novobiocin (Table 2).

Because of the similarity in the response of HL-205 and HL-
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The percentages are the mean ± SE from at least three experiments. The concentrations were chosen on the basis that they resulted in less than 30% cell killing as determined by the trypan blue exclusion test. Coumermycin at 1 μM, m-AMSA at 0.05 μM, and camptothecin at 0.08 μM, which were less cytotoxic, did not induce these differentiation markers. The cell number is the number of viable cells after exclusion of the cells stained with trypan blue.

Table 2 Induction of differentiation markers and growth inhibition in HL-205 cells after 4 days of continuous treatment with topoisomerase inhibitors

The percentages are the mean ± SE from at least three experiments. The concentrations were chosen on the basis that they resulted in less than 30% cell killing as determined by the trypan blue exclusion test. Coumermycin at 1 μM, m-AMSA at 0.05 μM, and camptothecin at 0.08 μM, which were less cytotoxic, did not induce these differentiation markers. The cell number is the number of viable cells after exclusion of the cells stained with trypan blue.

Table 3 Inhibition of novobiocin- and PMA-induced differentiation markers by H-7 in HL-205 cells

The percentages are the mean ± SE from at least three experiments. H-7 at 20 μM was added 1 h prior to the 4-day treatment with either 1 nM PMA or 150 μM novobiocin.

Table 4 Induction of differentiation markers after 4 days of treatment with either PMA or novobiocin in HL-205 cells

The percentages are the mean ± SE from at least three experiments. Novobiocin at 150 μM was added 1 h prior to 0.3 nM PMA. ND, not determined.

525 cells to induction of differentiation by both PMA and novobiocin, and because PMA-induced differentiation is believed to involve activation of PKC (26, 28), we tested the ability of H-7, an inhibitor of protein kinases, including PKC (27), to suppress the expression of maturation markers evoked by these two inducers. Our results indicate that H-7 was indeed capable of reducing the novobiocin- and PMA-induced reactivity with the OKM1 antibody and the activity of NSE (Table 3), as well as the induced reactivity with the MoP-9 antibody and reduction of NBT (novobiocin only) by about 60% (data not shown). However, H-7 was not able to prevent the novobiocin-evoked growth inhibition and cytotoxicity in either HL-205 or HL-525 cells. In addition, our results also indicate that the induction of maturation markers (reactivity with OKM1 and NSE activity) by 150 μM novobiocin and 0.3 nM PMA at 4 days after treatment was additive. This novobiocin concentration did not prevent the induction of differentiation by a higher dose of PMA (1.0 nM), which by itself caused more than 95% of the cells to react positively with the OKM1 antibody (Table 4).

These studies implicate a protein kinase, most likely PKC, in the induction of cell differentiation by both PMA and novobiocin. However, this induction probably does not involve a direct effect of novobiocin on PKC because, unlike PMA, novobiocin did not compete for [3H]phorbol-12,13-dibutyrate binding to its HL-205 cellular receptors, nor did novobiocin activate purified PKC when this kinase was tested for histone H1 phosphorylation (28).

Activity of Topoisomerase I and II in HL-205 and HL-525 Cells. Because of the possibility that a common step in novobiocin- and PMA-induced differentiation is a reduction in either Topo II or Topo I activity, we tested these activities in untreated and inducer-treated HL-205 and HL-525 cells.

Analysis of the nuclear extracts from the untreated cells revealed that the extract from the differentiation-susceptible HL-205 cells exhibited a specific activity for Topo II of 5 × 10^3 units/mg protein, whereas the extract from the resistant HL-525 cells contained a 4-fold higher activity of 20 × 10^3 units/mg protein (Fig. 2). Unlike these differences in Topo II activity, the activity of Topo I was similar in the extracts from both cell
types, yielding an activity of $3.3 \times 10^5$ units/mg protein (Fig. 2).

Treatment of the HL-60 cell variants with 150 $\mu$m novobiocin caused a reduction in Topo II activity in the extracts from both HL-205 and HL-525 cells, with the reduction in the extract from the HL-205 cells being most prominent. After 6 h of treatment, the Topo II activity in the extracts from the HL-205 cells, but not the HL-525 cells, was reduced by 4-fold. A 24-h treatment resulted in an 8-fold reduction in Topo II activity in the extracts from the HL-205 cells but less than one-half of this reduction in the extract from the HL-525 cells (Fig. 3). In contrast, the response of Topo I activities in these extracts was similar in both cell types; these activities were reduced after 6 h of treatment by 2-fold and after 24 h by 6-fold (Fig. 4).

We examined the possible role of protein kinases in these events by analyzing the reduction in topoisomerase activities in the nuclear extracts from HL-205 cells treated with either novobiocin or PMA in the presence and absence of H-7. To ensure that the reduction in Topo II activity is an early event and is thus associated with the induction process, we tested the effects of novobiocin and PMA shortly after their incubation with the cells. This shorter duration of treatments required higher drug concentrations. Treatment of differentiation-susceptible HL-205 cells for only 30 min with either 500 $\mu$m or 2 $\mathrm{mm}$ novobiocin in the absence of H-7 resulted in a 2-fold and a 6-fold reduction of Topo II activity, respectively. A 2-fold reduction in Topo II activity was also observed after a 30-min treatment with 0.2 $\mu\text{M}$ PMA (Fig. 5). Little or no reduction in Topo II activity was observed after such treatments of the resistant HL-525 cells. The reductions in Topo II activity in the treated HL-205 cells, however, were prevented when the cells were preincubated with H-7 (Fig. 5). In contrast, H-7 did not markedly alter the Topo I activities in the nuclear extracts from HL-205 or HL-525 cells treated for 30 min with 2 $\mathrm{mm}$ novobiocin.

Phosphorylation of Topoisomerase II in HL-205 and HL-525 Cells. To study the involvement of protein kinases in modulating Topo II activity in the HL-205 and HL-525 cells, we incubated the cells with $\text{32}^\text{P}$ and immunoprecipitated the phosphorylated Topo II with a specific antibody and further separated the enzyme by SDS-polyacrylamide gel electrophoresis. The major fraction of the radioactivity was detected in the Topo II ($M_r$, 170,000) band. Densitometric scanning revealed that the level of phosphorylated Topo II from the HL-205 cells was four times lower than that from the HL-525 cells (Fig. 6). Treatment of the HL-205 cells with 0.2 $\mu\text{M}$ PMA for 30 min resulted in a 2-fold increase in the level of phosphorylated Topo II, while a treatment with 2 $\mathrm{mm}$ novobiocin caused an 8-fold decrease in the level of the phosphorylated enzyme (Fig. 6). These PMA- and novobiocin-evoked changes in the level of phosphorylated Topo II were prevented by pretreatment of the HL-205 cells with H-7 (Fig. 6). Treatment of HL-525 cells with either PMA

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**Fig. 2.** Topo I and Topo II activities in nuclear extracts of HL-205 or HL-525 cells. Serial dilutions of the nuclear extracts served as the enzyme source. A, Topo I activity was determined by converting supercoiled pUC 8 plasmid DNA (I) into its relaxed form (I rel). The plasmid contained also a small amount of the nicked form (II). The negative control (−) contained the reaction mixtures without the nuclear extract. The positive control (+) contained the reaction mixture with a sample of purified Topo I from calf thymus. The reaction mixtures included the following amounts of extract protein: Lane 1, 100 ng; Lane 2, 30 ng; Lane 3, 10 ng; Lane 4, 3 ng; Lane 5, 1 ng. B, Topo II activity was determined by converting knotted P4 phage DNA (K) to its unknotted form (L). The phage DNA preparation also contained a small amount of linear form (L). The negative control (−) contained the reaction mixture without the nuclear extract. The positive control (+) contained the reaction mixture with a sample of knotted P4 DNA. The reaction mixtures included the following amounts of extract protein: Lane 1, 800 ng; Lane 2, 400 ng; Lane 3, 200 ng; Lane 4, 100 ng; Lane 5, 50 ng. Results are representative of one of three similar but independent experiments.
or novobiocin did not result in a marked alteration in the level of phosphorylated Topo II.

To determine whether the differences in the levels of phosphorylated Topo II are due to either changes in the amount of this enzyme or a different degree of its phosphorylation, we estimated the amount of the enzyme following its immunoblotting with an anti-Topo II antibody. The results indicated the presence of similar Topo II levels in both HL-205 and HL-525 cells, as well as in HL-205 cells treated with PMA (Fig. 7). In contrast, treatment of the HL-205 cells with novobiocin re-
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Fig. 5. Topo II activity in nuclear extracts from untreated HL-205 cells (A) and from HL-205 cells treated for 30 min with either 2 mM novobiocin (B), or 0.2 μM PMA (C) in the absence or presence of 0.2 mM H-7. The cells were incubated with H-7 60 min prior to and during the treatment with either novobiocin or PMA. The negative (−) and the positive control (+) are as described in the legend of Fig. 2B. The reaction mixtures included the following amounts of extract protein: Lane 1, 800 ng; Lane 2, 400 ng; Lane 3, 200 ng; Lane 4, 100 ng; Lane 5, 50 ng. The results are representative of one of three similar but independent experiments.

resulted in a decrease in the amount of the M, 170,000 Topo II band, most likely because of its degradation through a M, 150,000 band (Fig. 7). This decrease in the amount of Topo II was also prevented by preincubation of the HL-205 cells with H-7 (Fig. 7).

These results suggest that the higher level of phosphorylated Topo II in HL-525 cells as compared to that in HL-205 cells and the increased level of phosphorylated enzyme in PMA-treated HL-205 cells results from an increase in Topo II phosphorylation. In contrast, the decrease in the level of phosphorylated enzyme in the novobiocin-treated HL-205 cells results from enzyme degradation, most likely mediated by a protein kinase-dependent protease.

DISCUSSION

In the present studies, we investigated the roles of Topo II and Topo I in regulating the growth and maturation of two human HL-60 leukemia cell variants (HL-205 and HL-525) that differ in their susceptibility to differentiation induced by PMA but not other chemical inducers (28). Our results indicated that HL-205 cells that are susceptible to differentiation induced by PMA are also susceptible to induction of differentiation by novobiocin. These results, and those from our other studies (which indicate that novobiocin does not inhibit PMA-induced differentiation but may rather act additively with PMA), are in contrast to those of Sahyoun et al. (42), who found that novobiocin inhibited the differentiation induced by phorbol-12,13-dibutyrate. This apparent contradiction may be because Sahyoun et al. (42) determined cell differentiation after only 11.5 h of treatment with the phorbol diester, an insufficient time period to detect commonly used maturation markers (17, 24, 25, 28, 30). Moreover, cell attachment, the only marker used in their study, is a common response of different blood cell types to treatment with phorbol diesters and may not necessarily be indicative of cell maturation (43, 44).
Topo II inhibitors such as coumermycin and m-AMSA were less effective than novobiocin in inducing differentiation in HL-205 cells. This reduced ability may be attributed to their excessive cytotoxicity; unlike novobiocin, coumermycin is highly cytotoxic to the HL-60 cells, and its cytotoxicity is detected at concentrations that are lower than those required to inhibit Topo II activity (45). The antitumor drug m-AMSA is a potent cytotoxic drug because of its ability to stabilize the double-strand DNA breakage that results from the interaction of Topo II with DNA (15, 46, 47), which may prevent the expression of genes critical for cell differentiation.

In addition to inducing differentiation in HL-205 cells, novobiocin and PMA were able to cause a reduction in the Topo II activities of the HL-205 cells within a short time (30 min); little or no reduction in this enzyme activity was found after treatment of the resistant HL-525 cells. This observation may be attributed to the dissimilar levels of Topo II activities found in the two cell types; Topo II activity in the nuclear extract from HL-205 cells is 4-fold lower than that in the nuclear extract from the HL-525 cells. However, in contrast, novobiocin reduced the Topo I activities to a similar degree in both HL-205 and HL-525 cells. Novobiocin also caused a similar reduction in the cell growth in both cell types. On the other hand, PMA, which does not inhibit the growth of HL-525 cells (28), also did not alter the Topo I level in these cells.

On the basis of these results, we suggest that the reduction of Topo II activity in HL-60 cells is associated with induction of differentiation, while the reduction in Topo I seems to be linked to inhibition of cell growth. This suggestion is further substantiated by results from our experiments with H-7, an inhibitor of protein kinases (27). In these studies, H-7 diminished both the induction of differentiation and the reduction in Topo II activities evoked by either novobiocin or PMA in the HL-205 cells. However, this inhibitor did not effectively prevent the novobiocin-elicted inhibition of cell growth or reduction in Topo I activities in either HL-205 or HL-525 cells.

The reduction in Topo II activity shortly (30 min) after treatment of the HL-205 cells with either novobiocin or PMA probably occurs through different modes of action. The novobiocin-evoked reduction in Topo II activity cannot be explained by a possible reduction in the intracellular level of ATP (48), because the assay used in our study to measure Topo II activity calls for an addition of exogenous ATP to the enzyme reaction (32). The novobiocin-evoked reduction in Topo II activity is most likely due to an enhanced enzyme hydrolysis. Our results have shown that 30-min treatment of the HL-205 cells with novobiocin resulted in a degradation of Topo II. The mechanism underlying this event may involve the interaction of novobiocin with Topo II, which causes the enzyme to change its conformation, thus rendering it more susceptible to a proteolytic attack. Fluctuations in Topo II levels during the cell cycle of chicken lymphoblastoid cells are also attributed in part to proteolysis of the enzyme (49). The activity of the hydrolytic enzyme(s) in the novobiocin-treated cells is most likely dependent on protein kinase activities because Topo II degradation in the HL-205 cells was prevented by preincubation with H-7. The PMA-induced reduction in Topo II involves a different mode of action because treatment of HL-205 cells with this inducer did not enhance degradation of Topo II. Recent studies have shown that Drosophila and Geodia (spomge) Topo II can be manipulated in vitro by phosphorylation with PKC (42, 50). Thus, PMA, a PKC activator (26), may inhibit Topo II activity through its effect on phosphorylation of the topoisomerase. Indeed, our studies have shown that PMA treatment of HL-205 cells results in an increase in Topo II phosphorylation, which is abrogated by pretreatment with H-7, a PKC inhibitor (27). The PKC activities found in HL-205 and HL-525 cells differ in a number of characteristics, including substrate specificities (51). It is thus possible that the types of PKCs that are found and activated by PMA in HL-205 cells may phosphorylate Topo II at sites that render this enzyme less active, while other types of PKC, such as that found in HL-525 cells, may phosphorylate Topo II at sites that make it more active. Another possibility is that HL-205 cells contain a Topo II inhibitor that is dependent on PKC activation. Thus, the increase in Topo II phosphorylation and the resulting increase in enzyme activity may be neutralized by activation of the Topo II inhibitor. A third possibility is that HL-205 and HL-525 cells each have a different type of Topo II, as described for m-AMSA-susceptible and -resistant cells (29), which may respond differently after phosphorylation.

In brief, our results suggest that a reduction in Topo II activity is an early event that is associated with induction of differentiation in HL-60 cells and perhaps other cell types. Furthermore, our studies raise the possibility that the antibiotic drug novobiocin, and perhaps some of its analogues, may become useful in the control of some types of malignant cells by inducing them to differentiate into nondividing mature cells.

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