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Interaction of DNA Intercalator 3-Nitrobenzothiazolo (3,2-*a*)quinolinium with DNA topoisomerases: A Possible-Mechanism for Its Biological Activity

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SUMMARY

There is multiple evidence linking the inhibition of DNA topoisomerases I and II with the cytotoxic effects of antitumor drugs such as camptothecin and the DNA intercalators, 4-(9-acridinylamino)methanesulfon-*m*-anisidine) (*m*AMSA) and Adriamycin. We have assessed the effect of the DNA intercalator 3-nitrobenzothiazolo(3,2-a)quinolinium (NBQ) on the DNA topoisomerase I and II activities. NBQ has no effect on the activity of purified topoisomerase I, whereas it induced purified topoisomerase II binding to DNA without inducing DNA scission. Above 30 μM, NBQ stimulated formation of double- and single-strand breaks mediated by topoisomerase II in plasmid DNA. Using the alkaline elution technique we determined that NBQ induced single-strand and DNA-protein-associated breaks in the human promyelocytic leukemia cell line HL-60. At sublethal concentrations (≤1 μM), NBQ induce HL-60 cells to differentiate. Topoisomerase II-mediated DNA cleavage induced by *m*AMSA was substantially reduced in NBQ-differentiated cells. Our data suggest that topoisomerase II could play a major role in the biological activity of NBQ *in vivo*.

Recent studies have demonstrated that a variety of antineoplastic drugs interfere with the function(s) of topoisomerases I and II, which are fundamental for cellular replication and division (1–6). Tewey *et al.* (7) demonstrated that mammalian DNA topoisomerase II was a target for a group of intercalative antitumor drugs. Topoisomerase II activity and content varies according to cell proliferation (8) and differentiation (9–11). In murine erythroleukemia cells differentiated with 5 mM hexamethylene bisacetamide, the topoisomerase II is reduced to 10% of that in proliferating cells (9). Dimethyl sulfoxide-induced differentiation transiently activates both topoisomerase I and II (10), whereas differentiation of HL-60 cells by phorbol-12-myristate involves a reduction in topoisomerase II activity (11).

NBQ (Fig. 1) has been shown to be an effective antitumor agent (12). Furthermore, NBQ associates with DNA by the mechanism of intercalation (13). We have also shown that NBQ blocks DNA, RNA, and protein synthesis in KB and Ehrlich tumor ascites cells with equal effectiveness (14). Moreover, NBQ significantly enhances the lens regeneration of the adult newt *Notophtalmus viridescens* (15). NBQ induces the promyelocytic leukemia cell line HL-60 to undergo differentiation along the myeloid pathway.[3] These differences in biological activity are remarkable in view of the evidence accumulated on the binding of NBQ to DNA. In the current study, we examined the effects of NBQ on DNA topoisomerases I and II. We used alkaline elution to determine the production of SSB and DPC in HL-60 cells and isolated nuclei. In addition, we studied *in vitro* the interaction of NBQ with purified DNA topoisomerases I and II. Our results indicate that NBQ interferes with the DNA topoisomerase II activity.

Materials and Methods

Chemicals and Supplies

NBQ was a gift of Dr. O. Cox, University of Puerto Rico (San Juan, Puerto Rico). Stock solutions of both NBQ and *m*AMSA (NCS-249992) were made in dimethyl sulfoxide at 10 mM and were kept frozen at 20° until further use. [^{3}H]Thymidine (1 Ci/mmol) and [^{14}C]thymidine (1 Ci/mmol) were from Amersham. Chemicals were obtained from commercial sources.

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ABBREVIATIONS: NBQ, 3-nitrobenzothiazolo[3,2-a]quinolinium; *m*AMSA, 4-(9-acridinylamino)methanesulfon-*m*-anisidide; SSB, single-strand breaks; DPC, DNA-protein cross-links; SDS, sodium dodecyl sulfate; DSB, double-strand breaks.

Cells

The promyelocytic leukemia cell line HL-60 was grown in suspension culture in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum. All media were supplemented with penicillin, streptomycin, and fungizone. Cells used were in the exponential growth phase and cell viability was determined by trypan blue exclusion. Nuclei were isolated from HL-60 cells at 0°, as described in previous works (16, 17).

Alkaline Elution

Alkaline elution procedures were performed essentially according to the method reported previously (2). Briefly, HL-60 cells were incubated overnight in either 0.02 μCi/ml [^{14}C]thymidine or 0.2 μCi/ml [^{3}H]-thymidine. After a 2-hr chase with fresh medium without label, [^{14}C]-thymidine-labeled HL-60 cells or isolated nuclei were incubated at 37° with NBQ or *m*AMSA at different concentrations, 1 hr for cells or 30 min for isolated nuclei. [^{3}H]Thymidine-labeled cells were irradiated with either 300 or 1000 rad and used as an internal standard.

For SSB, a mixture of ^{14}C-labeled cells or nuclei and ^{3}H-labeled cells or nuclei were filtered on polycarbonate filters in ice-cold phosphate-buffered saline and lysed with a solution containing 2% SDS, 0.025 M EDTA, and 0.5 mg/ml proteinase K, pH 10. Alkaline elution was carried out with a solution of tetrapropylammonium hydroxide with 20 mM EDTA, pH 12.1, and 0.2% SDS. Eluted fractions were collected and counts/vial were measured in a liquid scintillation counter. The elution kinetics of the treated cells were normalized as a percentage of the internal control [^{3}H]DNA elution in each filter. The kinetics of SSB resealing were determined by removing the drug and incubating the cells in drug-free medium for certain time periods, followed by determination of SSB. DNA DSB were measured as described by Bradley and Kohn (18).

DPC were measured by mixing treated or untreated ^{14}C-labeled cells with ^{3}H-labeled internal standard cells, which were then irradiated with 3000 Rad and deposited onto polyvinyl chloride filters. Cells were lysed with LS-10 solution (2 M NaCl, 0.2% sarkosyl, 0.04 M EDTA, pH 10). Filters were washed with 0.04 M EDTA, pH 10, and elution of DNA was performed with tetrapropylammonium hydroxide, 0.02 M EDTA, pH 12.1.

The filter binding assay of DNA-protein linking was performed essentially as described by Pommier *et al.* (19). Briefly, 20 ng of [^{14}C]-DNA were incubated in 0.2 ml of nucleus buffer, with topoisomerase I or II, in the presence or absence of different concentrations of NBQ for 20 min at 37°. Reactions were stopped with 20 mM Na_2EDTA, pH 10, at 4° and filtered through a polyvinyl chloride filter. Filters were washed with LS-10 solution followed by 20 mM Na_2EDTA, pH 10. The effluents, filter washes, and filter were collected separately and processed for radioactivity determination.

Assessment of in vitro topoisomerase activity

Enzyme preparation. Topoisomerase II was purified from calf thymus, whereas topoisomerase I was purified from human placenta. Purification of enzymes was performed according to the procedure described previously (20).

Decatenation activity. Topoisomerase II activity was quantified by testing the ability of purified calf thymus topoisomerase II to decatenate kinetoplast DNA isolated from *Trypanosoma cruzi* (20, 21). Reactions were for 30 min at 37° in 20 μl containing 20 mM Tris·HCl, pH 7.9, 80 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM EDTA, 0.5 mM dithiothreitol, 15 μg/ml bovine serum albumin, 0.2 μg of enzyme, and 0.1 μg of kinetoplast DNA. Reactions were stopped with the addition of 5 μl of 1% SDS, 20% glycerol, 0.05% bromophenol blue. Samples were electrophoresed through a 2% agarose gel.

Cleavage activity. The ability of topoisomerases I and II to cleave DNA was tested with pGFC5A as substrate, as described previously (20, 21). The reaction mixture contained, in 20 μl, 20 mM Tris·HCl, pH 7.9, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM EDTA, 0.5 mM dithiothreitol, 10 μg/ml bovine serum albumin, 0.1 μg of pGFC5A, and 0.5 μg of the purified topoisomerases I or II. The reaction mixtures were incubated for 10 min at 37° and stopped with 5 μl of 5% SDS, 4 mg/ml proteinase K, 0.02% bromophenol blue, 20% glycerol. The mixture was incubated 45 min at 50° and electrophoresed in a 1.2% agarose gel.

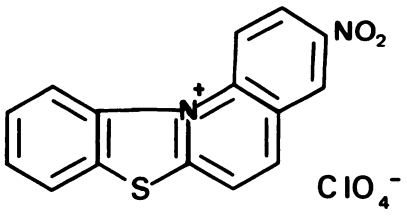

Fig. 1. Structure of NBQ.

Results

Induction of DNA SSB by NBQ in HL-60 cells. We measured the formation of DNA SSB in HL-60 cells, following a 1-hr incubation with various concentrations of NBQ, using the alkaline elution technique (Fig. 2). NBQ produced a low frequency of breaks. The number of SSB produced in HL-60 cells rose to a maximum of 176 rad-equivalents at a concentration of 100 μM and decreased concomitantly with increasing drug concentration, yielding a bell-shaped curve. Under the same conditions, 0.1 μM *m*AMSA, the established intercalative topoisomerase II inhibitor, induced 98 rad-equivalents, close to published values (22).

The reversibility of the intercalator-induced breakage was evaluated for NBQ. HL-60 cells were incubated for 1 hr with 100 μM NBQ, washed, and incubated in drug-free medium for an additional 1 hr at 37° before the production of SSB was tested. The number of SSB induced by NBQ on HL-60 cells was reduced to control levels after drug removal, demonstrating a rapid reversal of the lesions induced by NBQ (data not shown).

In order to obviate effects due to cellular transport (23), SSB

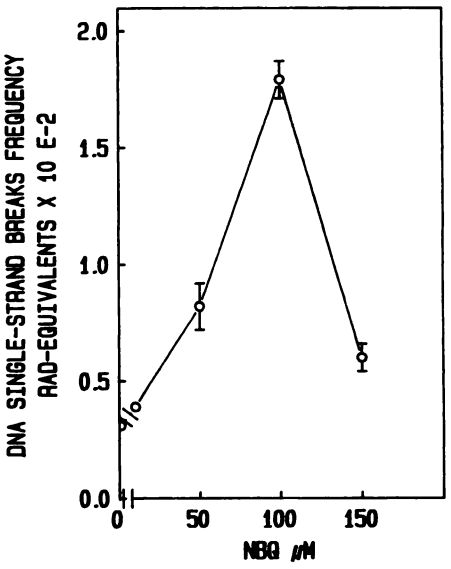

Fig. 2. NBQ-induced SSB in the HL-60 cell line. SSB was quantified using the alkaline elution technique developed by Kohn and co-workers (2). HL-60 cells were incubated for 1 hr at 37°, with or without increasing concentrations of NBQ. Results are expressed as the amount of X-irradiation (rad-equivalent) that produces random DNA breaks. *Points* are the mean of at least three separate experiments with a deviation of less than 10%.

were also measured in HL-60 isolated nuclei. Table 1 shows the frequency of SSB obtained. The number of SSB produced by NBQ in HL-60 isolated nuclei was 10 times higher than that obtained in whole cells. The dose-response curve is again bell-shaped and the peak maximum is displaced to lower concentrations (10 μM; 2335 rad-equivalents). On the other hand, 1 μM *m*AMSA produced 1982 rad-equivalents, which again is comparable to published results (22).

Induction of DNA DSB by NBQ in HL-60 cells. DNA DBS were measured in HL-60 cells after treatment with various concentrations of NBQ, ranging from 0.1 to 150 μM. None of the concentrations tested induced detectable DBS (results not shown). Thus, DNA breaks induced by NBQ are mostly SSB, similar to *m*AMSA (2, 24).

Induction of DPC by NBQ in HL-60 cells. The frequency of DPC produced by NBQ in HL-60 cells is depicted in Fig. 2. The production of DPC follows a bell-shaped curve and the maximum is achieved at 10 μM NBQ, with 186 rad-equivalents.

The apparent SSB/DPC ratio obtained for NBQ at various concentrations is shown in Table 2. The ratio increased from 0.25 at 10 μM NBQ up to a maximum of 1.7 at high NBQ concentrations (100 μM). The median ratio of SSB/DPC for NBQ was 0.77. The 50% inhibitory concentration of NBQ and *m*AMSA following a 60-min exposure of HL-60 cells to each drug is 10 and 0.9 μM, respectively (results not shown). Consequently, the cytotoxicity of NBQ toward HL-60 cells corresponds to concentrations where maximal production of DPC was achieved.

Production of DPC after inducing HL-60 cells to differentiate with NBQ. NBQ is able to induce differentiation of HL-60 cells toward the myeloid pathway at concentrations lower than the IC_{50} (2.7 μM in continuous cultures). Also, it has been suggested that topoisomerase II activity is associated with the differentiation status of the cells (9–11). Thus, we measured the drug-induced production of DPC in nuclei from HL-60 cells previously induced to differentiate for 5 days with 1 μM NBQ. Prior studies demonstrate that, at this stage, 80% of HL-60 cells are differentiated to metamyelocytes and neutrophils.[3] The production of DPC induced by 1 μM *m*AMSA was quantified in NBQ-induced HL-60 nuclei and compared with those produced in nuclei of untreated HL-60 cells (Table 3). The DPC formed were reduced to 36%, as compared with those in control nuclei. Similarly, when nuclei of NBQ-induced cells were incubated further with 1 μM NBQ for 1 hr, they formed 28% of the DPC produced by the same drug concentration in the control nuclei (Table 3). These results suggest that the differentiation of HL-60 cells induced by NBQ is accompanied by a decrease in the drug-induced (*m*AMSA or NBQ) topoisomerase II-induced DNA cleavage.

In an effort to gain additional insight on the production of DNA breaks by NBQ *in vivo*, we studied the interaction of NBQ with purified DNA topoisomerases I and II *in vitro*.

Effect of NBQ on the DNA-binding activity of topoisomerases I and II. The covalent binding of DNA topoisomerases I and II to [^{14}C]λDNA in the presence of different concentrations of NBQ was determined by the filter binding assay (Table 4). The drug-stimulated DNA-enzyme covalent binding was assessed based on the increased binding of DNA to the filter after detergent treatment to displace noncovalent complexes. The capacity of purified human topoisomerase I to bind DNA was not increased by NBQ at the concentrations tested. On the other hand, the DNA-binding capacity of purified calf thymus topoisomerase II was stimulated by NBQ. Maximum binding was achieved at 1 μM and was unchanged with increasing drug concentration.

Effect of NBQ upon the cleavable complex formation. NBQ was tested for its ability to stimulate DNA cleavage *in vitro*, using purified calf thymus DNA topoisomerase II. As shown in Fig. 4, plasmid DNA pGFC5A was treated with

TABLE 1

SSB induced by NBQ and *m*AMSA on isolated nuclei of HL-60 cells

Nuclei of HL-60 cells were incubated with or without drug for 30 min at 37°. Drugs were removed and SSB were measured by alkaline elution as described in Materials and Methods. Results are expressed in rad-equivalents and are the mean ± SEM of at least three separate experiments.

Drug	Concentration	SSB
	μM	*rad-equivalents*
NBQ	1	1603 ± 100
	10	2335 ± 112
	50	1879 ± 79
	100	1680 ± 123
	150	996 ± 238
*m*AMSA	1	1982 ± 289

TABLE 2

Relationship between SSB and DPC induced by NBQ

Values are mean ± 1 SD of at least three independent determinations in duplicate.

NBQ	SSB	DPC	SSB/DPC
μM	*rad-equivalents*		
10	40 ± 1	169 ± 5	0.24
50	82 ± 10	107 ± 4	0.77
100	175 ± 8	103 ± 5	1.70

TABLE 3

DPC in isolated nuclei of HL-60 cells induced to differentiate by NBQ

Drug	Concentration	DPC		Induced / Control
		Control[a]	NBQ-induced[b]	
	μM	*rad-equivalents*		%
NBQ	1	2922	747	28
*m*AMSA	1	4745	1686	36

[a] HL-60 cells were incubated for 5 days, nuclei were isolated, and the formation of DPCs was measured by alkaline elution after a 1 hr exposure to NBQ or *m*AMSA.
[b] NBQ (1 μM) was added to HL-60 cells and, after 5 days, nuclei were isolated and resuspended in fresh buffer. Then they were additionally treated with *m*AMSA or NBQ for 1 hr at 37°. Then, DPCs were measured using alkaline elution at pH 12 without proteinase K. Results are the mean of at least two experiments in duplicate.

TABLE 4

Effect of NBQ and *m*AMSA on the DNA topoisomerases I and II binding to λ DNA

Reaction mixture contained 260 ng of topoisomerase I or II and 20 ng of ^{14}C-λ DNA with different concentrations of the drugs in 0.2 ml of nucleus buffer. Mixtures were incubated for 20 min at 37°. Covalent complex formation was assayed by filter binding, as described in Materials and Methods. Values are the mean ± 1 SD of duplicate determinations.

Drug	Concentration	Covalent binding	
		Topoisomerase I	Topoisomerase II
	μM	%	
None		9.8 ± 0.1	16.2 ± 0.7
NBQ	100	9.5 ± 0.0	25.0 ± 0.5
	50	9.1 ± 0.1	28.4 ± 0.2
	10	8.9 ± 0.3	24.9 ± 0.1
	1	10.2 ± 0.1	25.0 ± 0.0
	0.1	9.6 ± 0.2	15.9 ± 0.3
*m*AMSA	10	3.2 ± 0.1	28.0 ± 0.2

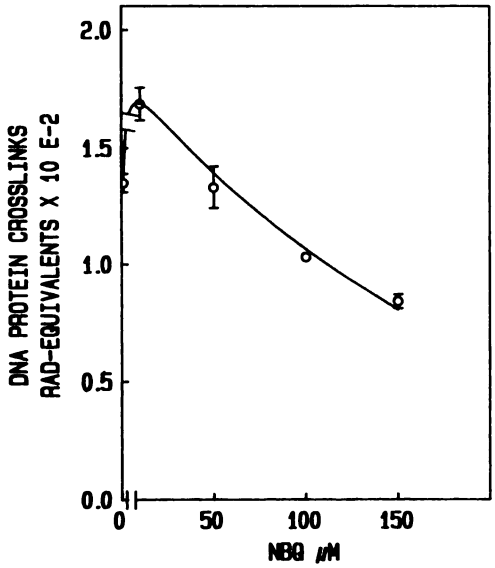

Fig. 3. NBQ-induced DPC in the HL-60 cell line. DPCs were quantified using the alkaline elution technique without proteinase K. HL-60 cells were incubated for 1 hr at 37° with or without increasing concentrations of NBQ. *Points* are the mean of at least two independent experiments in duplicate.

increasing concentration of NBQ from 1.9 to 250 μM. As the concentration was increased, the closed circular form (form I) was converted to the linear form III and to the nicked form II. NBQ stimulated DNA cleavage by DNA topoisomerase II but, contrary to *m*AMSA, high drug concentrations were necessary to stimulate the cleavage activity of topoisomerase II. At high concentrations of NBQ (250 μM) (Fig. 4, *lane A*), the plasmid DNA is completely converted to small fragments of DNA migrating as a smear.

The cleavage activity of topoisomerase I was also assayed in the presence of NBQ at the same range of concentrations. The cleavage activity of topoisomerase I was not stimulated by NBQ (results not shown).

Effect of NBQ on the decatenating activity of topoisomerase II. The decatenating activity of topoisomerase II on the kinetoplast DNA network is a specific double-stranded DNA passing activity of topoisomerase II that the topoisomerase I is unable to perform (25). To confirm that NBQ can inhibit the strand-passing catalytic activity of DNA topoisomerase II, we assessed the decatenating activity of purified calf thymus topoisomerase II in the presence of NBQ. As shown in Fig. 5, the decatenating activity of purified calf thymus topoisomerase II was inhibited by NBQ at high concentrations (125 μM) (Fig. 5, *lane C*) corresponding to the concentration that stimulated the cleavable complex formation (Fig. 3, *lane C*).

Discussion

Our results from *in vitro* studies using purified enzymes suggest that topoisomerase II was a target of NBQ action but topoisomerase I was not. The effect of NBQ on purified topoisomerase II decatenating and cleavage activity was dose related and was expressed at unusually high concentrations (Figs. 4 and 5). Whereas cleavage activity is expressed above 30 μM NBQ, the 50% inhibition of decatenating activity was achieved at 125 μM. Under the same conditions, *m*AMSA, a well known intercalator and inhibitor of topoisomerase II, induces DNA cleavage at 1 μM and inhibits 50% decatenating activity at 25 μM (results not shown). However, binding experiments have shown that topoisomerase II is bound to DNA by NBQ at concentrations lower than those necessary to stimulate the cleavable complex formation. Maximum covalent binding was achieved at 1 μM and was not modified with increasing drug concentration (Table 4). These results suggest that, at low concentrations, NBQ is able to bind topoisomerase II to DNA in an intermediate complex whose conformation differs from the cleavable complexes proposed for other topoisomerases inhibitors.

The cleavable complex was detected in HL-60 cells by alkaline elution. The SSB elution curve was bell-shaped, with a maximum at 175 ± 8 rad-equivalents for 100 μM NBQ. A rapid reversion of the lesions induced was observed after NBQ was removed from the cells. These results are consistent with previous reports on the reversibility of DNA scission described for other topoisomerase II inhibitors (2).

The DNA cleavage induced by NBQ was enhanced in isolated nuclei, where the maximum activity (2335 ± 112 rad-equivalents) was induced by a 10 times lower concentration of NBQ (10 μM). The most likely explanation for this enhanced activity when compared with the cleavage activity in whole cells is the accessibility of the drug to the nuclear DNA. These results might also suggest that intracellular metabolism of the drug was not required to induce the production of DNA scission. Thus, the absence of the barrier presented by the cell membrane that limits the influx of the drug into the cell could be associated with the high number of breaks in isolated nuclei.

The bell-shaped effect elicited by NBQ on the SSB as a

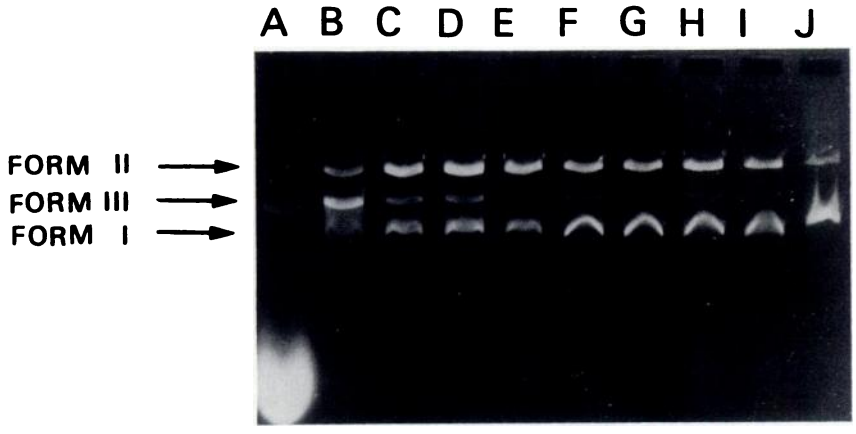

Fig. 4. *In vitro* stimulation by NBQ of the topoisomerase II DNA cleavage reaction. The DNA cleavage reaction was done as described in Materials and Methods, without or with increasing concentrations of NBQ. *Lane J*, plasmid DNA control (0.1 μg). *Lane I*, plus calf thymus topoisomerase II (200 ng). *Lanes A–H*, same as *lane I* plus 250, 175, 126, 84, 63, 32, 16, or 7.9 μM NBQ, respectively.

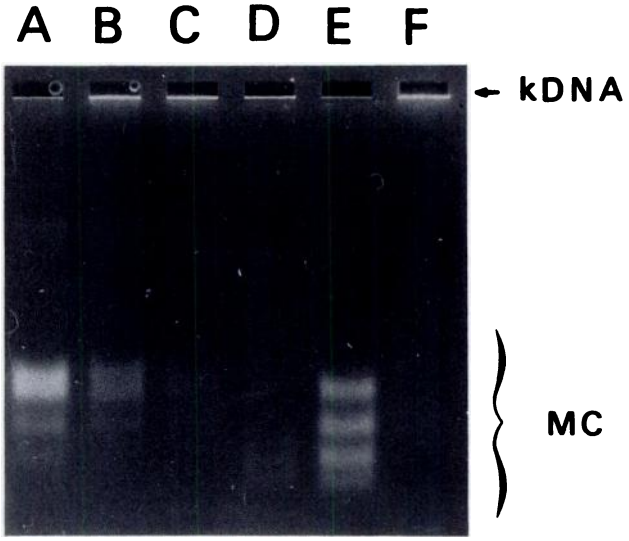

Fig. 5. Inhibition by NBQ of the decatenation reaction catalyzed by calf thymus DNA topoisomerase by calf thymus DNA topoisomerase II. The decatenation reaction was done as described in Materials and Methods. *Lane F*, control kinetoplast DNA (0.1 μg). *Lane E*, kinetoplast DNA plus topoisomerase II (50 ng). *Lanes A–D*, same as *lane E* plus 62.5, 125, 250, or 500 μM NBQ, respectively. Inhibition of 50% of the reaction is observed in *lane B* for 125 μM NBQ.

function of concentration was not reproduced in purified systems. Adriamycin and ellipticine derivatives are known to produce this effect in cellular and purified systems (7, 17, 19). This effect has been attributed to an altered binding complex induced by high concentrations of the drug that might distort the DNA-enzyme complex (7, 17, 19). Hence, we propose that these differences in activity of NBQ might be attributed to the interaction of topoisomerase II with NBQ at sites in the chromatin structure that are known to be modified (26). Such a chromatin structure might react with NBQ and topoisomerase II in a quite different manner to purified DNA *in vitro*.

NBQ produced DPC with the same bell-shaped effect as the SSB. However, the maximum DPC frequencies were obtained at a lower concentration of NBQ (10 μM) than the SSB. It has been shown previously that the SSB/DPC ratio induced by intercalators is approximately 1 (2, 27, 28), suggesting a direct association between each intercalator-induced strand break and the DPC formed. At low concentrations, NBQ induces more DPC than SSB (Table 2). These results are consistent with our observations *in vitro*, which show that NBQ stimulates the formation of DNA-topoisomerase II complexes without the induction of DNA scission. Formation of these complexes without associated DNA scission has not been observed previously with other intercalating antitumor drugs. Such complexes might not only contribute to the cytotoxicity of NBQ but also explain some of its distinct properties.

NBQ is able to differentiate HL-60 cells, a nonlethal effect, at doses that, although they do not induce a significant number of DNA breaks, facilitate binding of topoisomerase II to DNA. This phenomenon has not been observed previously with other intercalating agents. Recent studies demonstrated that the differentiation agents dimethyl sulfoxide and retinoic acid induced the formation of DNA strand breaks early in the differentiation of HL-60 cells (29). Therefore, the DPC formed at sublethal doses of NBQ might be related to the DNA strand breaks that are produced at the onset of cellular differentiation (29).

The frequency of total DPC induced by *m*AMSA in NBQ-differentiated HL-60 cells was one third of those produced in parallel undifferentiated cell cultures. The reduction in topoisomerase II activity assayed by drug-induced cleavage has been associated with the differentiated phenotype (9, 10, 29). We propose that the differentiated state of the HL-60 cells induced by NBQ could lead to a decrease of the topoisomerase II content of the cell, as previously described for other differentiation inducers (10, 11). Alternatively, we cannot exclude the possibility that the differentiation process induced by NBQ could lead to a modification of the chromatin structure, rendering less efficient the drug-induced topoisomerase II cleavage activity.

In conclusion, our results suggest that topoisomerase II might participate actively in the cytotoxic effect of NBQ by the formation of protein-associated DNA cleavage, similar to other intercalators. In addition, our results suggest that at low concentrations NBQ might induce DNA-topoisomerase II complexes without associated DNA scission. We propose that the differentiating ability of NBQ might be related to the formation of those complexes.

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References

1. Wang, J. C. DNA topoisomerases. *Annu. Rev. Biochem.* **54**:665–697 (1985).
2. Zwelling, L. A., S. Michaels, L. C. Erickson, R. S. Ungerleider, M. Nichols, and K. W. Kohn. Protein-associated deoxyribonucleic acid strands breaks in L1210 cells treated with the deoxyribonucleic acid intercalating agents 4′-(9-acridinylamino) methanosulfon, *m*-anisidine, and Adriamycin. *Biochemistry* **20**:6553–6563 (1981).
3. Ross, W. E., D. L. Glaubiger, and K. W. Kohn. Protein-associated breaks in cells treated with Adriamycin and ellipticine. *Biochim. Biophys. Acta* **519**:23–30 (1978).
4. Long, B. H., and A. Minocha. Inhibition of topoisomerase II by VP 16-213 (etoposide) and VM 26 (teniposide) and structural congeners as an explanation of *in vivo* DNA breakage and cytotoxicity. *Proc. Am. Assoc. Cancer Res.* **24**:321 (1983).
5. Hsiang, Y. H., R. Heitzberg, S. Hecht, and L. F. Liu. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.* **260**:14873–14878 (1985).
6. Trask, D. K., and M. T. Muller. Stabilization of type I-topoisomerase-DNA covalent complexes by actinomycin D. *Proc. Natl. Acad. Sci. USA* **85**:1417–1421 (1988).
7. Tewey, K. M., G. L. Chen, E. M. Nelson, and L. Liu. Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. *J. Biol. Chem.* **259**:9182–9187 (1984).
8. Chow, K.-C., and W. A. Ross. Topoisomerase-specific drug sensitivity in relation to cell cycle progression. *Mol. Cell. Biol.* **7**:3119–3123 (1987).
9. Bodley, A. L., H.-Y. Wu, and L. F. Liu. Regulation of DNA topoisomerase during cellular differentiation. *Natl. Cancer Inst. Monogr.* **4**:31–35 (1987).
10. O'Leary Bartus, J., S.-M. Mong, J. F. Zimmerman, F. H. Drake, K. B. Tan, C. K. Mirabelli, M. R. Mattern, and S. T. Crooke. Topoisomerases I and II: increase in intracellular activity at the onset of differentiation of human V937 cells. *Proc. Am. Assoc. Cancer Res.* **29**:121 (1988).
11. Constantinou, A. I., C. Henning-Chubb, and E. Haberman. Novobiocin and phorbol 12-myristate 13-acetate-induced differentiation of human leukemia cells is associated with inhibition of topoisomerase II activity. *Proc. Am. Assoc. Cancer Res.* **29**:137 (1988).
12. Cox, O., H. Jackson, V. A. Vargas, A. Báez, J. I. Colón, B. C. González, and M. de León. Synthesis and biological activity of benzothiazolo and benzoxazolo (3,2-*a*) quinolinium salts. *J. Med. Chem.* **25**:1378–1381 (1982).
13. Báez, A., F. A. González, D. Vázquez, and M. J. Waring. Interaction between a 3-nitrobenzothiazolo(3,2-*a*)quinolinium antitumor drug and deoxyribonucleic acid. *Biochem. Pharmacol.* **32**:2089–2094 (1983).
14. Casiano-Torres, C. A., and A. Báez. Effects of the antitumor drugs 3-nitrobenzothiazolo(3,2-*a*)qinolinium and fagaronine on nucleic acid and protein synthesis. *Biochem. Pharmacol.* **35**:679–685 (1986).
15. González, F. A., M. Llende, A. Báez, and J. R. Ortiz. The antitumor drug 3-nitrobenzothiazolo(3,2-*a*)quinolinium chloride (NBQ): effects on lens regeneration and interaction with DNA and *Notophtalmus viridescens*. *Differentiation* **36**:125–129 (1987).

16. Filipiski, J., and K. W. Kohn. Ellipticine-induced protein-associated DNA breaks in isolated L1210 nuclei. *Biochim. Biophys. Acta* **608**:280–286 (1982).
17. Pierson, V., A. Pierre, Y. Pommier, and P. Gros. Production of protein-associated DNA breaks by 10-[diethylaminopropylamino]-6-methyl-5 *H*-pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline in cultured L1210 cells and in isolated nuclei: comparison with other topoisomerase II inhibitors. *Cancer Res.* **48**:1404–1409 (1988).
18. Bradley, M. O., and K. W. Kohn. X-ray induced DNA double-strand break production and repair in mammalian cells as measured by neutral filter elution. *Nucleic Acid Res.* **7**:793–809 (1979).
19. Pommier, Y., J. K. Minford, R. E. Schwartz, L. A. Zwelling, and K. W. Kohn. Effects of the intercalators, 4-(9-acridinylamino) methanesulfon-*m*-anisidide (*m*AMSA) and 2-methyl-9-hydroxyellipticinium on topoisomerase II mediated DNA strand cleavage and strand passage. *Biochemistry* **24**:6410–6416 (1985).
20. Riou, J. F., M. Gabillot, M. Philipe, J. Schrevel, and G. Riou. Purification and characterization of *Plasmodium berghei* DNA topoisomerases I and II: drug action, inhibition of decatenation and relaxation and stimulation of DNA cleavage. *Biochemistry* **25**:1471–1479 (1986).
21. Douc-Rasy, S., A. Kayser, J. F. Riou, and G. Riou. ATP-independent type II topoisomerase from trypanosomes. *Proc. Natl. Acad. Sci. USA* **83**:7152–7156 (1986).
22. Bakic, M., M. Beran, B. S. Andersson, L. Silberman, E. Estey, and L. A. Zwelling. The production of topoisomerase II-mediated DNA cleavage in human leukemia cells predicts their susceptibility to 4-(9-acridinylamino)methane-sulfon-*m*-anisidide (*m*-AMSA). *Biochem. Biophys. Res. Commun.* **134**:638–645 (1986).
23. Deleted in proof.
24. Pommier, Y., R. E. Schwartz, K. W. Kohn, and L. A. Zwelling. Formation and rejoining of deoxyribonucleic acid double strand breaks induced in isolated cell nuclei by antineoplastic intercalating agents. *Biochemistry* **23**:3194–3201 (1984).
25. Marini, J. M., K. G. Miller, and P. T. Englund. Decatenation of kinetoplast DNA by topoisomerases. *J. Biol. Chem.* **255**:4976–4679 (1980).
26. Riou, J. F., M. J. Vilarem, C. J. Larsen, and G. Riou. *In vivo* stimulation by antitumor drugs of the topoisomerase II induced cleavage sites in c-*myc* protooncogene. *Biochem. Biophys. Res. Commun.* **137**:154–160 (1986).
27. Pommier, Y., R. E. Schwartz, L. A. Zwelling, and K. W. Kohn. Effects of DNA intercalating agents on topoisomerase II induced DNA strand cleavage in isolated mammalian cell nuclei. *Biochemistry* **24**:6406–6410 (1985).
28. Kohn, K. W., R. A. Ewig, L. E. Erickson, and L. A. Zwelling. Measurement of strand breaks and crosslinks by alkaline elution, in *DNA Repair: A Laboratory Manual of Research Techniques* (E. C. Freidberg and P. C. Hanawalt, eds.). Mercel Dekker, New York, 379–401 (1981).
29. Farzanch, F., R. Meldrum, and S. Shall. Transient formation of DNA strand breaks during the induced differentiation of human promyelocytic leukemic cell line, HL-60. *Nucleic Acids Res.* **15**:3493–3501 (1987).
30. Deleted in proof.

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