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Catalytic Inhibition of DNA Topoisomerase II by *N*-Benzyladriamycin (AD 288)

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ABSTRACT. *N*-Benzyladriamycin (AD 288) is a highly lipophilic, semi-synthetic congener of doxorubicin (DOX). Unlike DOX, which stimulates double-stranded DNA scission by stabilizing topoisomerase II/DNA cleavable complexes, AD 288 is a catalytic inhibitor of topoisomerase II, capable of preventing topoisomerase II activity on DNA. The concentration of AD 288 required to inhibit the topoisomerase II-catalyzed decatenation of linked networks of kinetoplast DNA was comparable to that for DOX. However, AD 288 did not stabilize cleavable complex formation or stimulate topoisomerase II-mediated DNA cleavage. In addition, AD 288 inhibited the formation of cleavable complexes by etoposide in a concentration-dependent manner. Human CCRF-CEM cells and murine J774.2 cells exhibiting resistance against DOX, teniposide, or 3′-hydroxy-3′-deaminodoxorubicin through reduced topoisomerase II activity remained sensitive to AD 288. These studies suggest that AD 288 inhibits topoisomerase II activity by preventing the initial non-covalent binding of topoisomerase II to DNA. Since AD 288 is a potent DNA intercalator, catalytic inhibition is achieved by prohibiting access of the enzyme to DNA binding sites. These results also demonstrate that specific substitutions on the aminosugar of DOX can alter the mechanism of topoisomerase II inhibition. BIOCHEM PHARMACOL **60**;11:1621–1628, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. topoisomerase II; *N*-benzyladriamycin; anthracyclines; catalytic inhibition

Eukaryotic type II DNA topoisomerases (topoisomerase II) are essential nuclear enzymes that regulate DNA topology. Topoisomerase II is crucial for DNA replication, transcription, and changes in chromosome structure that are associated with mitosis [1]. Several classes of antitumor compounds, including anthracyclines, epipodophyllotoxins, and aminoacridines, target topoisomerase II and interfere with its normal activity, resulting in cell cycle arrest and apoptosis [2]. Two general mechanistic classes of topoisomerase II-inhibiting drugs have been described: (i) topoisomerase II "poisons" that stabilize DNA/topoisomerase II cleavable complexes and stimulate double-stranded DNA cleavage, and (ii) catalytic inhibitors that prevent the interaction of topoisomerase II with DNA [3]. The specific mechanism of inhibition by members of each class of drugs depends, at least in part, upon the DNA intercalative strength of the drugs. As reviewed by Pommier *et al.* [4], non-intercalating or weakly intercalative topoisomerase II poisons, such as etoposide (VP-16) and teniposide (VM-26), increase the formation of cleavable complexes with increasing drug concentration. Intercalative poisons, such as DOX,† daunorubicin, epirubicin, *m*-amsacrine, mitoxantrone, and ellipticine, produce net increases in cleavable complexes at low concentrations, but inhibit topoisomerase II binding to DNA at very high concentrations [5]. Catalytic inhibitors decrease cleavable complex formation by reducing the binding of topoisomerase II to target DNA. In this regard, intercalating and non-intercalating catalytic inhibitors function differently. Intercalating drugs, such as aclarubicin, appear to modify DNA duplex topology in a manner that prevents topoisomerase II from achieving the initial non-covalent complex with DNA [6]. Non-intercalating catalytic inhibitors, such as the bisdioxopiperazines, bind directly to topoisomerase II and lock the enzyme in a closed clamp conformation following the DNA religation step [7]. Regardless of the mechanism, catalytic inhibitors tend to share specific experimental characteristics: (i) inhibition of topoisomerase II activity without the significant stabilization of topoisomerase II/DNA cleavable complexes [3], (ii) inhibition of the formation of cleavable complexes in the presence of topoisomerase II poisons [8, 9], and (iii) circumvention of drug resistance conferred by decreased topoisomerase II activity [10–12]. Although significant structural variation is found among the drugs in both classes of topoisomerase II inhibitors, discrete structural modifications can alter the mechanism of topoisom-

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† *Abbreviations*: DOX, doxorubicin (Adriamycin®); kDNA, kinetoplast DNA; and MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

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erase II inhibition, or eliminate inhibition altogether. Among the anthracycline topoisomerase II inhibitors, for example, substitution of the C-10 carboxymethyl of aclarubicin with a hydroxyl is reported to alter the mechanism of activity from catalytic inhibition to cleavable complex stabilization [13]. The addition of acylester side chains of at least 5 carbons reduces topoisomerase II inhibition through reduced binding to DNA [14]. These studies indicate that modifications of specific sites on the anthracycline chromophore have profound effects on the characteristics of topoisomerase II inhibition. In this report, we show that *N*-benzylation of the aminosugar of DOX to yield *N*-benzyladriamycin (AD 288) results in the formation of a new and potent inhibitor of topoisomerase II that satisfies the criteria for an intercalative catalytic inhibitor.

MATERIALS AND METHODS

Drugs and Chemicals

AD 288 was prepared according to previously described procedures [15, 16]. VP-16, aclarubicin, and MTT were obtained from the Sigma Chemical Co. All drugs were dissolved in DMSO. Purified human topoisomerase II and kDNA were purchased from TopoGEN, Inc. Topoisomerase II was maintained as aliquots at −80°. [^{3}H]Thymidine (20 Ci/mmol) and [^{14}C]leucine (50 mCi/mmol) were purchased from NEN™ Life Sciences.

Cell Culture and Drug Cytotoxicity Assay

CCRF-CEM human lymphocytic leukemia cells and the teniposide-resistant CEM/VM-1 cells exhibiting altered topoisomerase II activity [17] were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. J774.2 murine macrophage-like cells and the 3′-hydroxy-3′-deaminodoxorubicin-resistant clone 17 (C17), also exhibiting reduced topoisomerase II activity, were maintained as described previously [18]. Cytotoxicity assays were performed using the MTT assay [19]. J774.2 cells were seeded in 96-well microtiter plates at an initial density of 2.5 x 10^4 cells/mL and were exposed continuously to drug for 72 hr before the addition of MTT and dimethylformamide/SDS solubilization buffer. CEM cells were seeded at a density of 2.5 x 10^5 cells/mL and exposed to drug for 1 hr, then washed extensively, and incubated in drug-free medium for 24 hr prior to the addition of MTT. The IC_{50} values (drug concentrations that inhibited cell proliferation by 50%) represent the means of at least three independent determinations, each performed in triplicate.

Topoisomerase II Decatenation Assay

Topoisomerase II activity was measured based upon the decatenation of kDNA from the insect trypanosome *Crithidia fasciculata* [20]. Briefly, 4 U of purified human topoisomerase II, 80 ng of catenated kDNA, and drug dissolved in DMSO were combined in reaction buffer (50 mM Tris–HCl, pH 8.0, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 30 μg/mL of BSA, and 0.5 mM ATP) and incubated at 37° for 45 min. The reaction was terminated with 1.0% sarkosyl, 0.005% bromphenol blue, and 5% glycerol. Topological forms of kDNA were resolved by 1% agarose gel electrophoresis in Tris-borate/EDTA buffer and stained with 0.5 μg/mL of ethidium bromide after electrophoresis. DMSO concentrations in each reaction were maintained at 0.8% by the addition of equal volumes of serially diluted drug stocks so as not to produce solvent-mediated inhibition of topoisomerase II activity. Conversion of catenated kDNA to unlinked monomer kDNA was quantified by densitometric analysis with an Alpha Imager 2000 (Alpha Innotech Corp.).

Cleavable Complex Formation Assay

CEM cells were suspended in fresh medium at a density of 5×10^5 cells/mL for 3 hr at 37° followed by incubation in [^{3}H]thymidine (1.2 μCi/mL) and [^{14}C]leucine (0.1 μCi/mL) for 18 hr. Then cells were treated with drug for 30 min at 37°. In combined drug treatment experiments, AD 288 or DOX was added 10 min prior to VP-16 and co-incubated with VP-16 for 30 min. Drug/topoisomerase II interaction was terminated by the addition of a 2X buffer to a final concentration of 1.25% SDS, 5 mM EDTA, and 0.4 mg/mL of sonicated salmon sperm DNA. Cell lysates were passed 20 times through a 21-gauge syringe needle, and then heated to 65° for 15 min. Protein with covalently bound DNA was precipitated by the addition of KCl to 0.1 M and cooling on ice for 5 min. The precipitate was pelleted at 12,000 *g* for 8 min at 4°. The pellet was washed in 1 mL of 10 mM Tris–HCl, pH 8.0, 100 mM KCl, 1 mM EDTA, and 0.1 mg/mL of sonicated salmon sperm DNA and heated to 65° for 15 min with repeated mixing. Then the sample was cooled on ice for 5 min, and pelleted at 12,000 *g* for 8 min. The precipitate was washed two additional times by the same method. Following the final wash, the precipitate was dissolved in 500 μL water and heated to 65° for 15 min with intermittent mixing. Radioactivity was quantified by scintillation counting with results expressed as cpm (^{3}H/^{14}C).

Statistical Evaluation

Statistical analyses were performed by unpaired two-tailed Student's *t*-test accompanied by the *F*-test. Differences were considered significant when the *P* value was less than 0.05.

RESULTS

Topoisomerase II Inhibition by AD 288

As we have shown previously by fluorescence microscopy, the addition of the *N*-benzyl moiety onto the primary amine at C-3′ of DOX to produce AD 288 (Fig. 1) does not alter the nuclear localization of the anthracycline [21]. This is consistent with topoisomerase II as a principal cellular

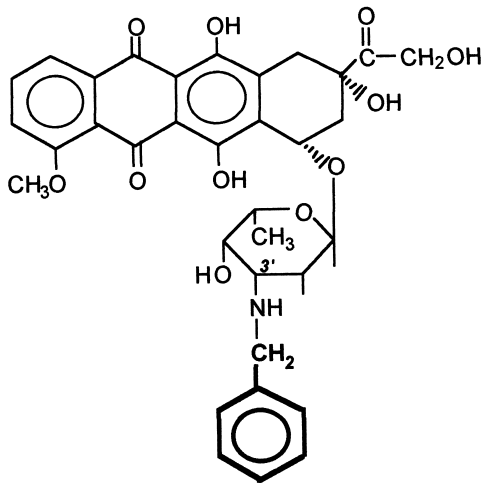


FIG. 1. Chemical structure of AD 288. The benzyl group (in bold) replaces a single hydrogen on the C-3′ amine of DOX.

target site for AD 288 [22]. Accordingly, AD 288 inhibited topoisomerase II-catalyzed decatenation of kDNA, a linked aggregate of 2.5 kb monomer DNA circles. Catenated kDNA is of sufficient size so as not to enter a 1% agarose gel. Topoisomerase II, through double-stranded DNA cleavage and religation, decatenated kDNA into unlinked monomers that migrated rapidly into an agarose gel (Fig. 2A). In assays using purified human topoisomerase II, AD 288 inhibited kDNA decatenation in a concentration-dependent manner with an IC_{50} value of 21 nM, i.e. the drug concentration required to inhibit the generation of monomer kDNA by 50% (Fig. 2B). In identical assay conditions, DOX exhibited an IC_{50} value of 54 nM, while aclarubicin, an anthracycline catalytic inhibitor of topoisomerase II, also inhibited kDNA decatenation in a concentration-dependent manner, with an IC_{50} value of 7 nM. VP-16, a non-intercalating topoisomerase II poison, showed less effective inhibition, with an IC_{50} value of 16 μM (data not shown). Based upon these results and previous results from our laboratories comparing DOX and AD 288 inhibition of topoisomerase II in nuclear lysates of murine cells [23], we concluded that AD 288 is an inhibitor of topoisomerase II activity with a potency that is comparable to other anthracycline topoisomerase II inhibitors.

Lack of Concentration-Dependent Cleavable Complex Stabilization by AD 288

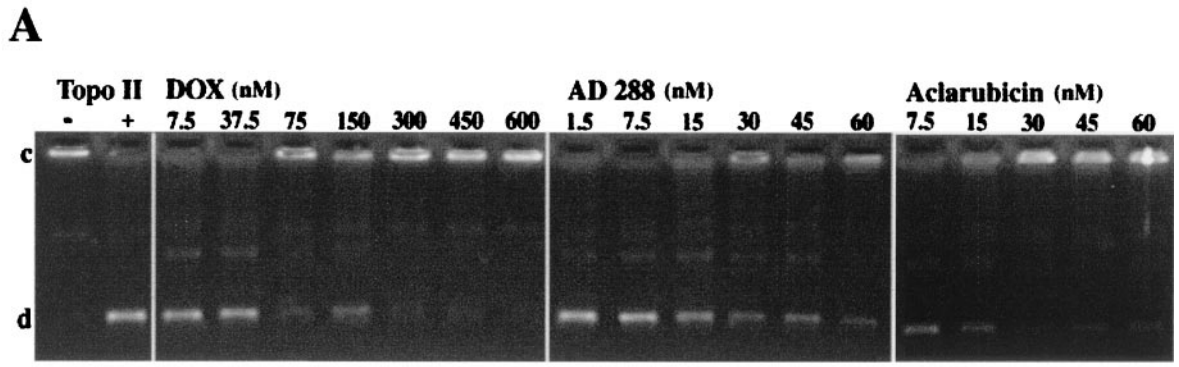
Despite the potency of topoisomerase II inhibition as determined by the kDNA decatenation assay, AD 288 failed to produce a concentration-dependent increase in DNA cleavage that is characteristically observed with topoisomerase II poisons (Fig. 3). The stabilization of topoisomerase II/DNA cleavable complexes by topoisomerase II poisons is quantifiable by the isolation of ^{3}H-labeled DNA covalently bound to precipitated ^{14}C-labeled protein from drug-treated cells. DOX produced a modest but clearly concentration-dependent increase in the amount of precipitable cleavable complexes up to 1.6-fold over DMSO-treated cells (Fig. 3). Over the same concentration range, no increase in cleavable complex formation was observed with AD 288 (Figs. 3 and 4A). Intracellular accumulation of AD 288 is much more rapid than that of DOX; AD 288 attains steady-state levels within 30 min, whereas DOX achieves comparable levels following up to 6 hr of drug exposure [21]. Therefore, treatment of CEM cells with 80 μM DOX, for 30 min to achieve equimolar intracellular DOX levels compared with 5 μM AD 288, produced a 2.1-fold increase in cleavable complex formation over control, or 1.7-fold more cleavable complex formation than 5 μM AD 288. AD 288, at concentrations up to 10 μM, did not inhibit topoisomerase I, based upon inhibition of topoisomerase I-mediated relaxation of supercoiled plasmid (data not shown). Aclarubicin, despite its catalytic inhibition of topoisomerase II activity, produced an overall concentration-dependent increase in cleavable complex formation up to 5 μM (data not shown), which can be ascribed to the additional action of aclarubicin as a topoisomerase I poison capable of producing topoisomerase I/DNA cleavable complexes [24, 25]. These results indicate that AD 288 does not stimulate concentration-dependent DNA cleavage at concentrations that exceed those capable of inhibiting the decatenation activity of topoisomerase II.

Inhibition of VP-16 Stabilization of Cleavable Complexes by AD 288

Catalytic inhibitors can impede cleavable complex formation by the topoisomerase II poison VP-16 through concentration-dependent inhibition of topoisomerase II binding to DNA [8]. Consequently, when CEM cells were pretreated with increasing concentrations of AD 288 for 10 min prior to the addition of 10 μM VP-16 for 30 min, a concentration-dependent decrease in cleavable complex formation was observed (Fig. 4A). Pretreatment with 0.5 μM AD 288 decreased cleavable complex formation by greater than 50%, whereas 3 μM AD 288 reduced cleavable complex formation to the basal level measured by treatment of CEM cells with 3 μM AD 288 alone. In contrast, DOX was unable to inhibit VP-16-mediated cleavable complex formation in a concentration-dependent manner at concentrations up to 100 μM (Fig. 4B).

Circumvention of Altered-Topoisomerase II-Mediated Drug Resistance by AD 288

Previous studies of CEM/VM-1 cells have demonstrated that despite significant levels of resistance to topoisomerase II poisons, these cells remain sensitive to both intercalating and non-intercalating catalytic inhibitors of topoisomerase II [10, 12, 26]. It would be predicted, then, that CEM/VM-1 cells would not be cross-resistant to AD 288. The cytotoxicity of AD 288 was measured in CEM/VM-1 selected for 72-fold resistance to VM-26 [17] and the cloned J774.2 murine macrophage-like cell line C17, selected for 11-fold



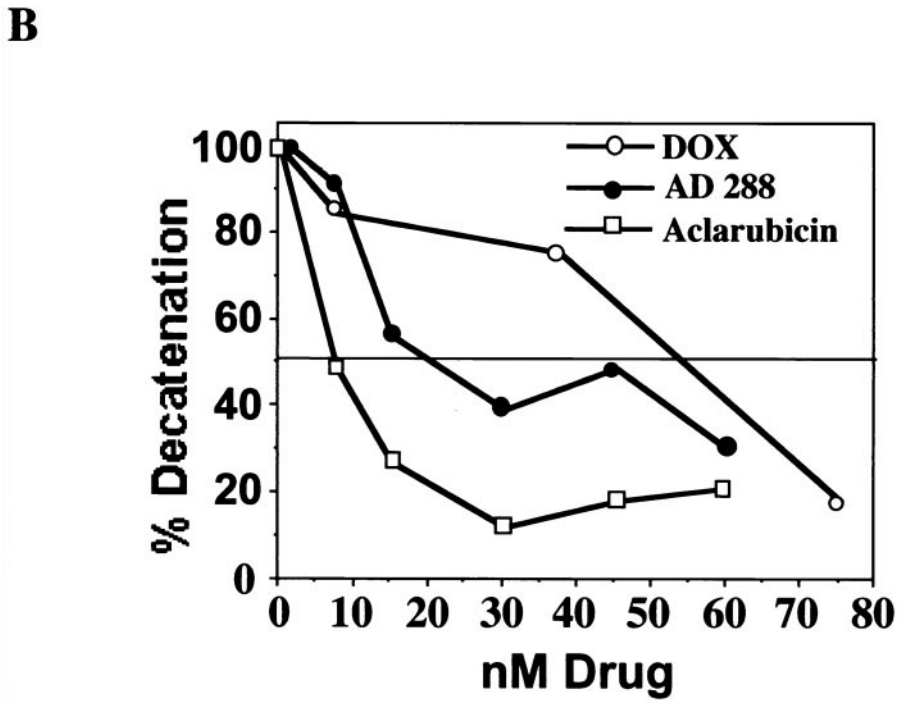


FIG. 2. Decatenation assay for topoisomerase II activity. (A) Eighty nanograms of catenated kDNA and drug dissolved in DMSO were combined in reaction buffer before incubation at 37° for 45 min. The reaction was terminated with 1.0% sarkosyl, 0.0005% bromphenol blue, and 5% glycerol. Topological forms of kDNA were resolved by 1% agarose gel electrophoresis in Tris-borate/EDTA buffer and stained with 0.5 μg/mL of ethidium bromide after electrophoresis. The concentration of each drug added to the reaction is indicated. Key: (−) kDNA incubated without topoisomerase; (+) kDNA incubated with topoisomerase II and without drug; (c) position of catenated kDNA; and (d) position of relaxed monomer kDNA. (B) Densitometric analyses of monomer kDNA generation plotted as percent decatenation of catenated substrate following incubation in the indicated concentrations of drug. Results presented are representative of three independent determinations.

resistance to the topoisomerase II poison hydroxyrubicin ([27]; Table 1). Following a 1-hr drug exposure and 24 hr growth in drug-free medium, CEM/VM-1 cells were 5.1- and 8.6-fold resistant to VP-16 and DOX, respectively, but exhibited no significant cross-resistance to AD 288. Similar results were observed following a 72-hr incubation in drug-free medium past initial drug exposure (data not shown). Likewise, following a 72-hr continuous exposure to drug, C17 cells were 4.5- and 6.0-fold resistant to VP-16 and DOX, respectively. However, C17 cells were not cross-resistant to either aclarubicin or AD 288. Cytotoxicity assays in which J774.2 and C17 cells underwent a 1-hr drug exposure followed by a 72-hr incubation in drug-free medium revealed that C17 cells were 1.5-fold more sensitive to AD 288 than were parental J774.2 cells (data not shown).

Based upon its intercalative potency [22], AD 288 likely inhibits topoisomerase II through direct binding to DNA rather than to enzyme. This mechanism is indicated by the results of kDNA decatenation assays in which the amount

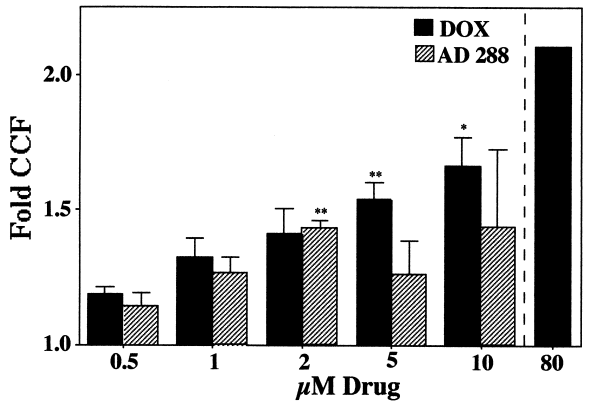


FIG. 3. Cleavable complex formation (CCF) analysis of AD 288. [^{3}H]Thymidine/[^{14}C]leucine dual-labeled CEM cells were treated with either DOX or AD 288 as described in Materials and Methods. The extent of drug-stabilized CCF is expressed relative to CCF in cells treated with the equivalent amount of DMSO only. Error bars represent the standard deviations of at least three determinations. Statistical analysis was performed by Student's *t*-test. *P* values: (**) 0.047; and (*) 0.0137.

TABLE 1. Cell proliferation analyses of CEM and J774.2 cells

	IC_{50}* (μM)		
	CEM	CEM/VM-1	R†
DOX	15.8 ± 0.2	136.0 ± 6.0	8.6
VP-16	32.7 ± 2.8	167.0 ± 3.2	5.1
AD 288	1.8 ± 0.1	2.0 ± 0.2	1.1

	IC_{50}‡ (nM)		
	J774.2	C17	R
DOX	79.4 ± 4.9	473.7 ± 39.3	6.0
VP-16	576.7 ± 17.7	2,570.0 ± 30.0	4.5
AD 288	29.7 ± 7.7	31.7 ± 8.2	1.1
Aclarubicin	14.9 ± 0.9	13.5 ± 1.5	0.9

* IC_{50}: Drug concentration that inhibits cell proliferation by 50% after a 1-hr drug exposure and continued incubation in drug-free medium for 24 hr. Values are the means ± SEM of at least three independent determinations, each performed in triplicate.

† R: Resistance factor: ratio of resistant cell line/parental cell line.

‡ IC_{40}: Drug concentration that inhibits cell proliferation by 50% after continuous exposure for 72 hr. Values are the means ± SEM of at least three independent determinations, each performed in triplicate.

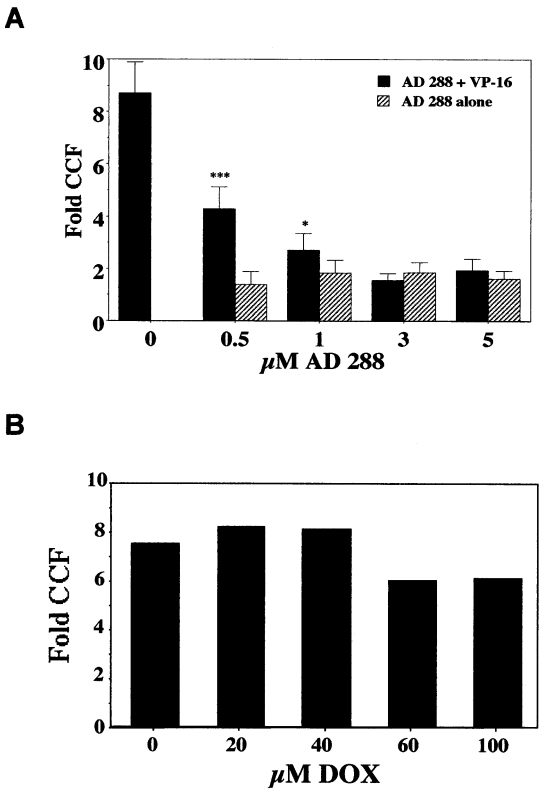


FIG. 4. Inhibition of VP-16-mediated cleavable complex formation (CCF) by AD 288. Metabolically labeled CEM cells were treated with 10 μM VP-16 for 30 min alone (panels A and B) or pretreated for 10 min with increasing concentrations of AD 288 (panel A) or DOX (panel B) prior to 30 min combined treatment with VP-16 and either AD 288 or DOX. CCF is expressed as described for Fig. 3. Error bars represent the standard deviations of at least three determinations. Statistical analyses were performed by Student's *t*-test to determine the significance of the differences between successive AD 288 concentrations. *P* values: (***) 0.002; and (*) 0.0109.

of DNA substrate was increased incrementally in order to produce an excess of substrate free of bound AD 288 (Fig. 5). Using a concentration of AD 288 that inhibits by greater than 90% the topoisomerase II-mediated generation of monomer kDNA from 25 ng of catenated substrate, increasing the amount of kDNA from 250 to 750 ng allowed monomer kDNA formation by topoisomerase II.

DISCUSSION

The results in this study demonstrated that the semisynthetic anthracycline AD 288 is a catalytic inhibitor of topoisomerase II based upon the ability of AD 288 to: (i) inhibit topoisomerase II activity without stimulating DNA strand scission in a concentration-dependent manner, (ii) inhibit the formation of stable cleavable complexes by VP-16 in a concentration-dependent manner, and (iii) circumvent drug resistance conferred by altered topoisomerase II activity. These results also demonstrated that modification of the C-3′ site on the aminosugar of DOX can influence the mechanism of topoisomerase II inhibition markedly. Previous studies have shown that modification of specific sites on both the chromophore and aminosugar

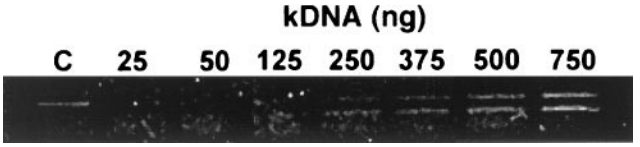


FIG. 5. Reversal of AD 288-mediated topoisomerase II inhibition by increasing amounts of DNA substrate. kDNA decatenation assays were performed as described for Fig. 2, except that the AD 288 concentration was maintained at 50 nM, and the amount of kDNA was increased as indicated. C = no drug (DMSO only) with 62.5 ng kDNA.

portions of DOX alters its interaction and degree of inhibition of topoisomerase II. Hydroxylation at C-11 or removal of the methoxy group at C-4 reduces the inhibitory potency of the resulting anthracycline, but does not alter the mechanism of topoisomerase II inhibition [28]. Acylester substitutions at C-14 greatly reduce topoisomerase II inhibition by abrogating drug/DNA binding and producing cytoplasmic localization of the anthracycline [21, 29, 30]. Jensen and co-workers [13] reported that substitution of the C-10 carboxymethyl on the A-ring of aclarubicin with a hydroxyl is sufficient to stimulate single-strand DNA breakage, which is undetectable with aclarubicin. However, stimulation of topoisomerase II-mediated DNA cleavage is not detectable with C-10 hydroxyl aclarubicin. It is not clear from these results whether C-10 hydroxyl substitution converted aclarubicin to a topoisomerase II poison or a topoisomerase I inhibitor.

The ability of DOX to stimulate topoisomerase II-mediated double-stranded DNA cleavage and the formation of stable cleavable complexes has been established previously [2, 22, 30, 31]. While the extent of detectable cleavable complex formation is characteristically low for DOX, compared with VP-16, as shown here and elsewhere [32], cleavable complex formation by DOX is, nevertheless, a concentration-dependent effect. *N*-Benzylation of DOX eliminated concentration-dependent cleavable complex formation activity without decreasing cellular cytotoxicity, probably through enhanced distortion of the DNA helix. The aminosugar of DOX extends outside the nucleotide base stack. Consequently, despite the addition of the *N*-benzyl moiety to the aminosugar, AD 288 has DNA intercalative strength comparable to that of DOX [22]. Therefore, it appears that the *N*-benzyl moiety would have little effect upon the characteristics of anthracycline intercalation into DNA, but may alter helix conformation through minor groove interaction. Distamycin and Hoechst 33258 bind to the minor groove of DNA and inhibit topoisomerase II catalytic activity, suggesting that the minor groove is a site of topoisomerase II binding, and that occupation of the minor groove by drug interferes with topoisomerase II binding [33, 34]. Footprinting analyses indicate that the bulky aminotrisaccharide moiety of aclarubicin lays in the minor groove of DNA [35], suggesting that the aminotrisaccharide may contribute to catalytic inhibition of topoisomerase II. The pendant *N*-benzyl group of AD 288 would, likewise, be expected to occupy the minor grove and, as such, to contribute to catalytic inhibition. The different mechanisms of topoisomerase II inhibition by DOX and AD 288 would indicate that within this class of anthracyclines, substitution on the sugar, rather than at C-10 on the chromophore, primarily determines the topoisomerase II inhibitory characteristics of the compound. Arcamone *et al.* [36], however, have described a 4′-linked aminodisaccharide analog of DOX that is a more effective topoisomerase II poison than DOX. Thus, the specific structure of the pendant aminosugar substitution appears to be critical in determining the mechanism of topoisomerase II inhibition.

The significance of the positive charge on the amine remains to be determined fully. The positive charge of the C-3′ amine of DOX helps stabilize binding to DNA through electrostatic interaction with the negatively charged phosphate backbone of DNA [37]. This interaction is not required for topoisomerase II inhibition, since 3′-hydroxy-3′-deaminodoxorubicin is also a potent topoisomerase II poison [27] and selects for resistance through decreased topoisomerase II activity [18]. However, the positive charge may be more important for catalytic inhibition, since a preliminary comparison of AD 288 with the uncharged congener 3′-*O*-benzyldoxorubicin (WP546; [5]) revealed that WP546 was not as effective as AD 288 in inhibiting topoisomerase II-catalyzed kDNA decatenation (Lothstein *et al.*, unpublished results). This is consistent with the 10-fold decrease in cytotoxicity of WP546 compared with AD 288 [38].

Our interest in AD 288 is based upon its identity as the principal cellular metabolite of *N*-benzyladriamycin-14-valerate (AD 198), a semi-synthetic compound with novel pharmacological properties and which does not inhibit topoisomerases I or II activities. Nevertheless, AD 198 is as cytotoxic as AD 288 and DOX, owing, at least in part, to its modulation of protein kinase C activity and the ability to circumvent several mechanisms of cellular drug resistance [14, 19, 23, 27, 38–41]. The cytotoxic properties of AD 198 are entirely distinct from those of AD 288, since apoptosis in cells treated with AD 198 can be detected prior to the conversion of AD 198 to AD 288 (Lothstein L, unpublished results). Nevertheless, we are currently investigating whether AD 198 can synergize the cytotoxic effects of AD 288, based upon the ability of non-cytotoxic levels of AD 198 to potentiate DOX-induced cytotoxicity [42].

Collateral sensitivity to ICRF-187 [12] and ICRF-159 [43] has been reported in cells whose resistance to topoisomerase II poisons is due to topoisomerase II down-regulation and reduced activity. In our own studies, collateral sensitivity to AD 288 was observed in a murine cell line with reduced topoisomerase II activity. Topoisomerase II activity is modulated, in part, by phosphorylation with several kinases, including protein kinase C, in a cell cycle phase-specific manner [44]. Reduced protein kinase C activity has been shown to be correlated with VP-16 resistance through hypophosphorylation of topoisomerase II and consequent reduced activity [45, 46], which can be reversed by the protein kinase C activator bryostatin [44]. Therefore, AD 198 cytotoxicity may be prolonged and potentiated as AD 198 is transformed from a protein kinase C modulator to a topoisomerase II catalytic inhibitor.

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