Enhanced topoisomerase II targeting by annamycin and related 4-demethoxy anthracycline analogues

Alex V. Trevino,1 Barbara A. Woynarowska,1 Terence S. Herman,1 Waldemar Priebe,2 and Jan M. Woynarowski1

1University of Texas Health Science Center, San Antonio, Texas and 2University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract
Targeting topoisomerase II (topo II) is regarded as an important component of the pleiotropic mechanism of action of anthracycline drugs. Here, we show that 4-demethoxy analogues of doxorubicin, including annamycin, exhibit a greater ability to trap topo II cleavage complexes than doxorubicin and some other 4-methoxy analogues. In leukemic CEM cells with wild-type topo II, annamycin induced substantial levels of topo II–mediated DNA-protein cross-links (15-37% of total DNA for 0.5-50 μmol/L drug), whereas doxorubicin-induced DNA-protein cross-links were marginal (0-4%). In CEM/VM-1 cells that harbor mutated, drug-resistant topo II, both 4-methoxy and 4-demethoxy drugs produced marginal DNA-protein cross-links. Annamycin, but not doxorubicin, formed topo II–mediated DNA-protein cross-links also in isolated CEM nuclei. In disparity with the unequal DNA-protein cross-link induction, both drugs induced comparable levels of DNA strand breaks in CEM cells. Compared with CEM, drug cytotoxicity against CEM/VM-1 cells was reduced 10.5- to 13.8-fold for 4-demethoxy analogues but only 3.8- to 5.5-fold for 4-methoxy drugs. Hence, growth inhibition by 4-demethoxy analogues seems more dependent on the presence of wild-type topo II. The enhanced topo II targeting by 4-demethoxy analogues was accompanied by a profound induction of apoptotic DNA fragmentation in leukemic CEM cells. Normal WI-38 fibroblasts, however, were markedly more resistant to annamycin-induced DNA-protein cross-links, apoptosis, and growth inhibition. The enhanced topo II targeting by 4-demethoxy doxorubicin analogues underscores the mechanistic diversity of anthracycline drugs. This diversity needs to be recognized as a factor in responses to drugs such as annamycin and doxorubicin. [Mol Cancer Ther 2004;3(11):1403–10]

Introduction
The anthracycline drugs such as doxorubicin and daunomycin are widely used anticancer agents effective against a variety of solid tumors and hematologic malignancies (1–3). Yet, their clinical utility is often compromised by tumor resistance and significant cardiotoxicity (1, 4, 5). Therefore, extensive efforts are conducted to develop new anthracycline analogues that would overcome these limitations (5–7).

Cellular effects of the anthracycline antibiotics involve an interplay of noncovalent DNA intercalation, formation of covalent DNA adducts, topoisomerase II (topo II) poisoning, and free radical effects on cellular membranes and DNA (2–4, 6–9). Intercalation between adjacent DNA bp distorts replication and/or transcription. Intercalation is also essential for the ability of anthracycline to trap topo II intermediates. Topo II catalyzes topological conversions of cellular DNA by forming a transient double-stranded break in DNA with the enzyme’s tyrosine residue covalently attached to the end of DNA strand in the break (3, 10). These intermediates, termed cleavable or cleavage complexes, are stabilized by various drugs, including many anthracyclines, which convert topo II to a cellular poison. Doxorubicin metabolism leads to free radical formation resulting in lipid peroxidation and oxidative DNA damage (11) and reactive drug intermediates that can bind covalently to DNA (12–17). Each of the mentioned pleiotropic activities of anthracyclines probably contributes to cell growth inhibition, although their relative significance may be cell type specific and may vary for different anthracycline analogues.

Although the contributions of individual routes remain unclear, topo II–mediated DNA lesions are often singled out as a major factor in antiproliferative effects of the anthracycline drugs (3, 6, 18). Unlike other topo II poisons, however, doxorubicin and daunomycin, the structural prototypes for the two main types of anthracyclines, trap very low levels of topo II cleavage complexes (19, 20), whereas topo II targeting by some other anthracycline drugs seems more significant (21). In particular, 4-demethoxy daunomycin analogues were found more efficient than daunomycin in trapping topo II cleavage complexes (6, 22, 23). Despite structural differences, daunomycin resembles doxorubicin in various biochemical properties. Hence, it seems likely that 4-demethoxy analogues of doxorubicin may also exhibit enhanced contribution of topo II targeting.
One of the promising 4-demethoxy doxorubicin congeners is annamycin (Fig. 1). Annamycin is a member of lipophilic doxorubicin analogues designed to circumvent multidrug resistance typical of doxorubicin (24–27). This study compared doxorubicin and annamycin and some other lipophilic 4-methoxy or 4-demethoxy doxorubicin analogues for their ability to target topo II and induce apoptosis. The results show that annamycin and several other 4-demethoxy analogues of doxorubicin exhibit a greater ability to trap topo II cleavage complexes than doxorubicin and some 4-methoxy analogues. Moreover, the findings suggest that the observed enhanced topo II targeting plays a role in the antiproliferative and lethal effects of annamycin and other studied 4-demethoxy analogues.

Materials and Methods

Drugs

The synthesis of annamycin and WP744 and related analogues has been described previously (28, 29). Drug stock solutions were made in water and stored at −20°C protected from light.

Cell Culture and Cytotoxicity Determinations

Leukemic CEM and CEM/VM-1 cells (from Dr. William T. Beck), normal fibroblast WI-38 cells (American Type Culture Collection, Rockville, MD), and normal prostate PrEC cells (Clonetics, San Diego, CA) were cultured as described elsewhere (30, 31). Drug cytotoxicity was measured by the standard assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; ref. 32) after a continuous exposure of cells to drugs for approximately three doubling times. The results are expressed as drug concentration inhibiting the net cell growth by 50% (GI50).

DNA-Protein Cross-links

DNA-protein cross-links in intact cells or isolated nuclei were assayed using the previously described K+/SDS precipitation technique (30, 33–35). The results are expressed as a percentage of total DNA coprecipitating with proteins (FP). The frequency of DNA-protein cross-links (f) was estimated based on a Poisson distribution according to Eq. (A):

\[
f = \frac{-\ln(1 - FP)}{\text{MW}_n/640}
\]

where MWn is number average molecular weight for DNA under the conditions of the assay and 640 is the average molecular weight of 1 bp. The MWn values of 5 × 10^7 and 4 × 10^7 Da for intact cells and isolated nuclei, respectively, were determined previously (30).

Single-Strand Breaks by Sedimentation Analysis

DNA single-strand breaks (SSB) were determined by sedimenting cellular DNA in 5% to 30% alkaline sucrose gradients in a SW41Ti rotor for 18 hours at 12,000 rpm. Other details of the assay were as described previously (36). The shifts in the fragment distribution profiles were used to estimate SSB frequency per bp (f) as described previously (36, 37) using Eq. (B):

\[
f = \frac{[(\text{MW}_n)_A/(\text{MW}_n)_B] - 1}{[(\text{MW}_n)_A/640]}
\]

where (MWn)_A and (MWn)_B are the number average molecular weights of control and drug-treated ^14C-radio-labeled DNA peaks, respectively. The value 640 represents the average molecular weight of 1 bp.

Apoptotic DNA Fragmentation

Apoptotic fragmentation assay that detects and quantifies a broad range of fragment sizes, from subnucleosomal particles up to at least ~150 kbp, has been described previously in detail (31, 34). Briefly, drug-treated [^14C]thymidine prelabeled cells were extracted in hypotonic buffer. Following centrifugation, released chromatin fragments were collected in the supernatants, whereas the pellets contained unfragmented DNA. The results are expressed as the percentage of the total DNA released into supernatants.

Figure 1. The structures of the studied 4-demethoxy and 4-methoxy anthracyclines.
In some experiments, cell growth and cell viability were determined under the conditions of apoptosis induction based on cell counts of parallel samples and trypan blue exclusion, respectively.

Results

Potent Induction of DNA-Protein Cross-links in Intact Cells by 4-Demethoxy Anthracyclines

Trapping of topo II cleavage complexes in cancer cells is manifested by the characteristic topo II–mediated lesions: rapidly induced DNA-protein cross-links. Such presumably topo II–mediated DNA-protein cross-links induced by the anthracycline analogues were examined in human leukemia CEM cells using the K+/SDS precipitation technique (20, 33–35, 38). In this assay, DNA coprecipitable with proteins is directly determined by the radioactivity in the protein/dodecyl sulfate pellet.

The results show a potent induction of DNA-protein cross-links by annamycin, WP794, WP769 (Fig. 2), and several other 4-demethoxy doxorubicin analogues (data not shown). The DNA-protein cross-link effects of annamycin and WP794, reaching ~30% of total DNA, are comparable with the effects of classic topo II poisons such as teniposide and amsacrine (33, 38). The DNA-protein cross-link induction by WP769 was somewhat less potent. Bell-shaped concentration dependence for this drug is indicative of self-inhibition at drug levels exceeding 2 μmol/L. Such an inhibition is common among intercalators (35) and is consistent with the greater DNA affinity of WP769 (due to positively charged amino sugar; Fig. 1) compared with analogues with hydroxyl in this position (39). Even this 4-demethoxy analogue, however, induced markedly higher levels of DNA-protein cross-links than the maximal effects of any 4-methoxy drugs examined (Fig. 2 and data not shown). For example, DNA-protein cross-links induced by a broad range of doxorubicin concentrations did not exceed 5%. Positive controls in these experiments (20 μmol/L of the classic topo II poison, amsacrine; 20 μmol/L of a nonspecific direct DNA-protein cross-link inducer, formaldehyde) resulted in 32.3 ± 0.9% and 90 ± 4% of DNA-protein cross-links, respectively. It needs to be noted that the observed differences in DNA-protein cross-link induction do not arise from any major differences in the overall cytotoxicity of the examined drugs. MIT assay after 48-hour incubation of CEM cells shows that doxorubicin and annamycin are essentially equitoxic, with GI50 values of 93 ± 7 and 92 ± 9 nmol/L, respectively.

Estimates of DNA-protein cross-link frequency in genomic DNA based on the K+/SDS data suggest that whereas annamycin and WP794 form 2 to 3 DNA-protein cross-links/10⁶ bp/μmol/L and WP769 forms ~1 DNA-protein cross-link/10⁶ bp/μmol/L, the effects of doxorubicin and WP744 are <0.05 DNA-protein cross-link/10⁶ bp/μmol/L. The markedly lower ability of doxorubicin to form topo II–mediated DNA-protein cross-links is fully consistent with the findings of previous studies using the same technique but other cell lines (19, 20).

In theory, the DNA-protein cross-link assay might detect not only topo-mediated but also topo-independent DNA-protein cross-links. Therefore, we did additional DNA-protein cross-link determinations in CEM/VM-1, a subline harboring altered (mutated) topo II that is inefficiently trapped by topo poisons (40). The results (Fig. 2B) confirm that DNA-protein cross-link induction by annamycin, WP769, and WP794 in CEM/VM-1 is dramatically reduced compared with DNA-protein cross-link levels in wild-type CEM cells. For example, the levels of DNA-protein cross-link induced by 2 μmol/L annamycin decreased from 25.3 ± 8.7% in CEM cells to 2.0 ± 0.1% in CEM/VM-1 cells. The abrogation of DNA-protein cross-links in cells with mutated topo II strongly corroborates that the substantial levels of DNA-protein cross-links formed by annamycin and other 4-demethoxy drugs in the wild-type CEM cells correspond indeed to trapped topo II intermediates.

Induction of SSBs

Because of the nature of the cleavage complexes, topo II–mediated DNA-protein cross-links are expected to be accompanied by similar levels of strand breaks. This
paradigm is met by the classic topo II drugs, which act by a single mechanism, such as epipodophyllotoxins and amsacrine (38, 41). Thus, we compared the ability of annamycin and doxorubicin to generate SSBs in CEM cells.

Sedimentation analysis in alkaline sucrose gradients shows that DNA from cells treated with either drug sediments more slowly than DNA from control cells (Fig. 3). A similar pattern was also observed for WP769. Such shifts in sedimentation are indicative of substantial strand breakage in cellular DNA and can be used to estimate SSB frequency. The estimates based on profiles like those in Fig. 3 suggest that annamycin produces 10.3 ± 3.8 SSBs/10^6 bp/μmol/L, which is somewhat higher but still fairly close to the estimated DNA-protein cross-link frequency for that drug. The induction of similar SSB levels by doxorubicin (10 ± 3 SSBs/10^6 bp/μmol/L) indicates an at least 40-fold greater ratio of SSB/DNA-protein cross-link for that drug. This disproportionate effect is consistent with the possibility that a major portion of SSB induced by doxorubicin in CEM cells might reflect other doxorubicin activities than trapping topo II intermediates.

**Topo II – Mediated Lesions in Isolated Nuclei**

To eliminate the possible interference of various cellular factors, such as drug uptake, metabolism, and free radical generation, we additionally examined the inherent potential of the drug to trap topo II cleavage complexes in isolated nuclei. These experiments confirmed that both annamycin and another 4-demethoxy analogue WP794 are potent DNA-protein cross-link inducers (Fig. 4). The magnitude of DNA-protein cross-link induction by either drug was slightly greater than DNA-protein cross-links generated by the equimolar level of the classic topo II poison amsacrine. In contrast to both 4-demethoxy anthracyclines, doxorubicin produced no detectable DNA-protein cross-links under these conditions (1.1 ± 5.1% and −1.5 ± 1.4% at 10 and 50 μmol/L, respectively).

DNA-protein cross-links represent a characteristic lesion for trapping not only topo II but also topo I intermediates. However, whereas topo II action requires ATP, topo I effects are ATP independent. DNA-protein cross-links by annamycin and WP794 were nearly eliminated in the absence of exogenous ATP and the presence of novobiocin, an agent that prevents ATP binding to topo II (Fig. 4). These results confirm that DNA-protein cross-links induced by these drugs are ATP dependent. Furthermore, the formation of topo-mediated DNA-protein cross-links, but not nonspecific DNA-protein cross-links mediated by covalent adduction of both DNA and proteins, can be prevented by the addition of a minor groove binding agent and a catalytic inhibitor of topo II distamycin (33, 35, 38, 41, 42).

DNA-protein cross-links by annamycin and WP794 are completely eliminated in the presence of distamycin. In contrast, direct nonspecific DNA-protein cross-links by formaldehyde are not affected by ATP removal or by distamycin addition. Collectively, the results in intact cells and isolated nuclei show that annamycin and other 4-demethoxy doxorubicin analogues exhibit enhanced topo II targeting compared with doxorubicin.

**Induction of Apoptosis and Cytotoxicity of 4-Demethoxy Analogues**

To address the possible connection between topo II targeting by anthracycline drugs and cell fate, we examined
the induction of apoptosis. A 24-hour exposure of CEM cells to 4-demethoxy anthracyclines annamycin, WP769, and WP794 and to 4-methoxy drugs doxorubicin and WP744 results in a strong apoptotic DNA fragmentation (Fig. 5A). This effect is seen at the concentrations of annamycin, WP769, and WP794 that are low (<1 μmol/L) but produce detectable DNA-protein cross-links (cf. Fig. 2). Significant apoptotic DNA fragmentation is observed at annamycin concentration as low as 0.1 μmol/L. In addition, treatment with 0.1 to 1 μmol/L annamycin results in decreased cell viability and net cell loss as indicated by negative values of relative cell growth (Fig. 5B). Both indices are characteristic of advanced apoptotic changes. In addition, annamycin-induced apoptosis is caspase mediated, as it is abrogated by a broad-spectrum caspase inhibitor, Z-VAD-fmk (data not shown). These observations are consistent with previous studies that documented strong proapoptotic effects of annamycin in other systems (43). Whereas doxorubicin requires somewhat higher concentrations for apoptosis induction, its lipophilic analogue, WP744, was as proapoptotic under these conditions as annamycin (Fig. 5; ref. 44).

Cytotoxicity of both 4-methoxy and 4-demethoxy drugs is greater in wild-type CEM cells than in the CEM/VM-1 subline with mutated topo II (Table 1). Importantly, however, the resistance factor for doxorubicin is only 3.8-fold compared with 13.8-fold for annamycin. A similar difference is seen for the lipophilic analogues 4-methoxy WP744 and 4-demethoxy WP769 with the resistance factor of 3.8 versus 10.5, respectively. Thus, the growth inhibitory effects of annamycin and WP769 are markedly more dependent on the presence of wild-type topo II than the effects of doxorubicin and WP744. This pattern is consistent with the idea that topo targeting plays a more significant role in the antiproliferative effects of the 4-demethoxy compounds than the 4-methoxy doxorubicin analogues.

**Topo II Effects and Apoptosis in Normal Cells**

DNA-protein cross-links and apoptosis induction by 4-demethoxy analogues were additionally measured in normal WI-38 cells to determine whether the enhanced topo II targeting is associated with any differentiation between tumor and normal cells. Under conditions identical to those used previously for cancer cells (Fig. 2), both annamycin and WP794 induce detectable topo II-mediated DNA lesions (DNA-protein cross-links), but the levels of these lesions are less profound than in tumor cells (Fig. 6A). Because the levels of cellular topo II strongly fluctuate with cell cycle, one simple explanation is that normal cells may, on average, have less topo II because of the reduced fraction of S-phase cells, although other factors might also contribute.

The reduced DNA-protein cross-links in cells are accompanied by marginal apoptotic responses to annamycin and WP794 (Fig. 6B). Similar low apoptosis was also noted for annamycin in normal prostate PrEC cells in contrast to strong apoptosis in prostate cancer LNCaP-Pro5 cells (data not shown). In addition, the cytotoxicity of annamycin, measured using the MTT assay, was 9.2-fold lower in normal WI-38 cells (GI_{50} 0.38 ± 0.09 μmol/L) than in wild-type CEM cells (GI_{50} 0.041 ± 0.012 μmol/L; Table 1). Although doxorubicin cytotoxicity was also lower in WI-38 cells, this reduction was only 2-fold (GI_{50} 0.25 ± 0.07 versus 0.124 ± 0.022 μmol/L in WI-38 and CEM cells, respectively).

**Discussion**

The ability of anthracycline drugs to trap topo II cleavage complexes is thought to be important for the biological properties of these clinically relevant drugs. However, anthracyclines differ from other topo poisons in that topo II

---

**Figure 5.** Induction of apoptosis in CEM cells by annamycin and other 4-demethoxy anthracyclines in leukemic CEM cells. A, apoptotic DNA fragmentation after 24-hour incubation with annamycin ( ), WP769 ( ), and WP794 ( ). Points, mean of one to three independent experiments carried out in duplicate; bars, SE. Dotted and dashed gray lines, profiles for doxorubicin and WP744, respectively, that were obtained under identical conditions and are replotted from ref. 44. B, relative cell growth (normalized cell growth corrected for time 0 values) and cell viability of CEM cells incubated 24 hours with annamycin. Points, mean of two experiments; bars, SE. Negative values of relative cell growth are indicative of net cell loss.
targeting is one of the several mechanistic facets by which these agents inactivate cancer cells. Therefore, anthracycline-induced DNA lesions may originate not only from topo II targeting but also from other mechanisms. Trapping of topo II cleavage complexes is expected to increase the fraction of DNA that is covalently linked to topo II molecules (topo II-mediated DNA-protein cross-links). However, some studies reported a marginal or insignificant induction of DNA-protein cross-links by doxorubicin in cancer cells (19, 20). Despite these and other ambiguities, the role of topo II targeting in the antiproliferative effects of anthracycline drugs is widely accepted. This report attempts to better define this role by (a) quantifying the ability of several doxorubicin analogues to induce topo II-mediated DNA-protein cross-links in cellular settings and (b) exploring the connection between topo II-mediated lesions and cell growth inhibition and apoptotic potential of these drugs.

Our findings show that the removal of the methoxy group at position 4 enhances the ability of doxorubicin analogues to trap topo II cleavage complexes. Unlike doxorubicin and 4-methoxy analogues, annamycin and other 4-demethoxy analogues consistently induce substantial levels of topo II-mediated DNA-protein cross-links in CEM cells and in isolated CEM nuclei. These DNA-protein cross-links have the following attributes expected for lesions that originate from drug trapping of topo II cleavable complexes: (a) rapid kinetics of formation, (b) coinduction of strand breaks, (c) dependence on ATP (abrogation by an ATP antagonist novobiocin), and (d) requirement for topoisomerase activity (abrogation by a topo II catalytic inhibitor distamycin). Finally, these DNA-protein cross-links require wild-type topo II, as the effect is nearly eliminated in CEM/VM-1 subline that harbors mutated, drug-resistant topo II.

The reported results validate enhanced topo II targeting by 4-demethoxy anthracyclines of the doxorubicin series in cellular settings. This finding corroborates and expands previous observations made by Capranico and coworkers, mainly in cell-free systems, that 4-demethoxy analogues in the daunomycin series are more efficient topo II poisons than daunomycin (6, 22, 23). Further investigations are needed to explain why and how the absence of the 4-methoxy group facilitates the more efficient stabilization of topo II cleavage complex. One clue is provided by a recent molecular modeling study, which indicated that the 4-methoxy group is not involved in any interactions needed for the stabilization of the ternary complex of doxorubicin/topo II/DNA (10). It seems conceivable that the absence of this bulky group might facilitate steric interactions of other components that are essential for the formation of the ternary complex.

Enhanced topo II targeting by annamycin and other 4-demethoxy analogues seems to play a proportionately greater role in the antiproliferative effects of these drugs than in the effects of 4-methoxy compounds. The comparison of drug effects in CEM and CEM/VM-1 cells shows that the cytotoxicity of 4-demethoxy analogues is more dependent on wild-type topo II than the cytotoxicity of 4-methoxy drugs. It needs to be emphasized, however, that the likely differences in the significance of topo II targeting for the overall cell kill do not imply that one type of

---

**Table 1. Cytotoxicity of 4-demethoxy and 4-methoxy anthracycline drugs against wild-type CEM leukemic cells with normal topo II and its variant CEM/VM-1 with mutated topo II**

<table>
<thead>
<tr>
<th>Drug type</th>
<th>Drug</th>
<th>CEM GI&lt;sub&gt;50&lt;/sub&gt; (µmol/L)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CEM/VM-1 GI&lt;sub&gt;50&lt;/sub&gt; (µmol/L)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Resistance factor&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Demethoxy</td>
<td>Annamycin</td>
<td>0.041 ± 0.012</td>
<td>0.570 ± 0.030</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>WP769</td>
<td>0.035 ± 0.012</td>
<td>0.372 ± 0.069</td>
<td>10.5</td>
</tr>
<tr>
<td>4-Methoxy</td>
<td>Doxorubicin</td>
<td>0.124 ± 0.022</td>
<td>0.684 ± 0.013</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>WP744</td>
<td>0.039 ± 0.004</td>
<td>0.148 ± 0.011</td>
<td>3.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Drug concentration inhibiting net cell growth by 50% determined using the MTT assay. Data are means ± SE from two to four independent determinations, each carried out with four to eight replicate samples.

<sup>b</sup>Ratio of the GI<sub>50</sub> values in CEM/VM-1 and CEM cells.

---

Figure 6. Topo II lesions and apoptosis induced by annamycin and WP794 in normal cells. A, topo II-mediated DNA-protein cross-links in normal WI-38 fibroblasts incubated for 30 minutes with annamycin (●) and WP794 (○). Dotted and dashed pale lines, tracings of annamycin and WP794 effects, respectively, in tumor CEM cells from Fig. 2. B, apoptosis induced after a 24-hour incubation of normal WI-38 fibroblasts with annamycin (●) and normal prostate PrEC cells with annamycin (●) and WP794 (○). Dotted and dashed pale lines, tracings of annamycin and WP794 effects, respectively, in tumor CEM cells from Fig. 5. Points, mean of two experiments; bars, SE.
anthracycline analogues is necessarily superior to the other. Both 4-methoxy and 4-demethoxy analogues are capable of apoptosis induction and inhibition of cancer cell proliferation.

The observed mechanistic differences need to be recognized as a likely factor in responses to drugs such as annamycin and doxorubicin. It might be advantageous to match drug mechanistic profiles to the molecular characteristics of a tumor to be treated. One may expect that enhanced apoptosis originating from topo II–mediated lesions would lead to therapeutic benefits in the treatments of tumors expressing high levels of topo II. Conversely, anthracyclines with lower contribution of topo II targeting to cell kill, such as 4-methoxy analogues, may be preferred in the case of tumors with low topo II activity. Moreover, a better understanding of the mechanistic diversity may be pertinent to drug selectivity for tumor cells. For instance, cardiotoxicity that is linked to the free radical mechanisms was reduced for annamycin compared with doxorubicin (45–47). Although the pleiotropic cytotoxicity of anthracycline drugs is well known, the underlying mechanistic diversity of specific anthracycline subtypes remains poorly understood. A systematic exploration of the aforementioned diversity is likely to help maximize the clinical potential of annamycin and other anthracycline drugs.

Acknowledgments

We thank Dr. Maryanne Herzig for cytotoxicity determinations and Dr. William T. Beck for CEM and CEM/VM-1 cells.

References

34. Wowarnowsky JM, Napier C, Koster SK, et al. Effects on DNA integrity
40. Wolverton JS, DankS MK, Schmidt CA, Beck WT. Genetic character-