Cancer Chemother Pharmacol (1997) 39: 455–461

ORIGINAL ARTICLE

Paul J. Smith · Nicola J. Blunt · Rodwige Desnoyers
Yvonne Giles · Laurence H. Patterson

DNA topoisomerase II-dependent cytotoxicity of alkylaminoanthraquinones and their N-oxides

Received: 12 August 1995 / Accepted: 14 August 1996

Abstract We studied the role of DNA topoisomerase II in the biological actions of a series of novel alkylaminoanthraquinones, including N-oxide derivatives designed as prodrugs liable to bioreductive activation in hypoxic tumour cells. Drug structures were based upon the DNA-binding anticancer topoisomerase II poison mitoxantrone with modifications to the alkylamino side chains. The agents included AQ4, 1,4-bis{[2-(dimethylamino)ethyl]amino}5,8-dihydroxy-anthracene-9,10-dione, and AQ6, 1{[2-dimethylamino)-ethyl]amino}4-{[2[(hydroxyethyl)amino]ethyl]-amino}5,8-dihydroxy-anthracene-9,10-dione, together with the corresponding mono-N-oxide (AQ6NO) and di-N-oxide (AQ4NO). The R_3N^+-O^- modification renders the terminal nitrogen group electrically neutral and was found to reduce AQ6NO or effectively abolish AQ4NO-DNA binding. Comparative studies were carried out using two SV40-transformed fibroblast cell lines, MRC5-V1 and AT5BIVA, the latter being a relative overproducer of DNA topoisomerase IIα. The inhibition of DNA topoisomerase II decatenation activity ranked according to DNA-binding capacity. A similar ranking was found for drug-induced DNA-protein cross-linking in intact cells, depending upon topoisomerase II availability. Inhibition of DNA synthesis in S-phase synchronized cultures ranked in the order of AQ6 > mitoxantrone ≫ AQ6NO and was independent of topoisomerase II availability. Cytotoxicity of acute 1-h exposures for all agents except the inactive AQ4NO was enhanced in the topoisomerase II-overproducing cell line. The results indicate an important role for enzyme targeting in anthraquinone action. However, DNA synthesis inhibition and cytotoxicity were greater than expected for AQ6, given its topoisomerase- and DNA-interaction properties, and parallel studies have provided evidence of an additional role for enhanced subcellular accumulation and nuclear targeting. The inactivity of AQ4NO and the retention of only partial activity of AQ6NO, allied with the effective topoisomerase II-targeting and high cytotoxic potential of their presumed metabolites, favour their use as prodrugs in tumour cells with enhanced bioreductive potential.

Key words Mitoxantrone · Anthraquinone prodrugs · Bioreduction · DNA intercalation · DNA topoisomerases

P.J. Smith (✉)
Department of Pathology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK

P.J. Smith · N.J. Blunt · R. Desnoyers
MRC Clinical Oncology and Radiotherapeutics Unit, MRC Centre, Cambridge, UK

R. Desnoyers
Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina, USA

Y. Giles · L.H. Patterson
Department of Pharmaceutical Sciences, De Montfort University, Leicester, UK

Introduction

The anthraquinones are a group of synthetic DNA-binding agents that are structurally related to the DNA-intercalating anthracycline antibiotics. A major anticancer agent within this group, mitoxantrone (mitozantrone; Novantrone) is used clinically in the treatment of non-Hodgkin's lymphomas, acute myeloid leukaemias and advanced breast cancer [18]. DNA topoisomerase II is a major target for diverse groups of anticancer DNA-intercalating agents [5, 7, 16, 22], this nuclear enzyme being vital for the efficient condensation/decondensation of chromatin and for the segregation of replicated daughter chromosomes at cell division [22]. The enzyme can actively alter the topology of DNA molecules by the introduction of transient double-strand breaks through which

Table 1 Side-chain composition of mitoxantrone and analogues

Compound	R_1	R_2
Mitoxantrone	$NHCH_2CH_2NCH_2CH_2OH$	$NHCH_2CH_2NCH_2CH_2OH$
AQ4	$NHCH_2CH_2N(CH_3)_2$	$NHCH_2CH_2N(CH_3)_2$
AQ6	$NHCH_2CH_2N(CH_3)_2$	$NHCH_2CH_2NCH_2CH_2OH$
AQ4NO	$NHCH_2CH_2N(O)(CH_3)_2$	$NHCH_2CH_2N(O)(CH_3)_2$
AQ6NO	$NHCH_2CH_2N(O)(CH_3)_2$	$NHCH_2CH_2NCH_2CH_2OH$

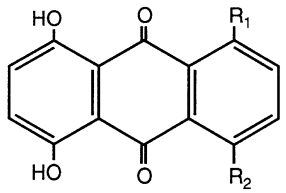

Fig. 1 General structure of the anthraquinones used in the present study (see Table 1 for details of the side-chain composition)

an intact helix can pass [22]. During this reaction the enzyme molecule forms a non-covalent protein-DNA complex that is in rapid equilibrium with a 'covalent cleavable' complex [7]. Mitoxantrone prevents re-ligation of transient double-strand DNA breaks by trapping the cleavable complex formed between DNA and topoisomerase II [1, 7]. It is thought that drug-DNA binding [3, 15, 20] and drug-induced DNA damage causes DNA synthesis inhibition [5], an arrest of cells in the G2 phase of the cell cycle and the progression through cell-death pathways.

Our aim is to improve selective chemotherapeutic activity by minimising systemic toxicity. One approach is to use prodrug design to harness tumour-cell selectivity for both the activation process and the mode of action of the metabolite. Accordingly, the basic concept pursued in the current study is that aliphatic tertiary amine N-oxides of cytotoxic anthraquinones are essentially deactivated DNA-binding agents that are capable of tumour selectivity through bioreductive conversion in hypoxic tumour cells [2, 9, 10, 12]. The activated agent would demonstrate persistent targeting of topoisomerase IIβ in quiescent cells or of topoisomerase IIα in cells opting to enter the cell cycle [4]. We report herein on the DNA topoisomerase II targeting of mitoxantrone analogues and their prodrug forms.

We have studied mitoxantrone and four related alkylaminoanthraquinones [11–14] comprising two N-oxides (AQ4NO and AQ6NO) and their putative immediate metabolites (AQ4 and AQ6), their structures being indicated in Table 1 and Fig. 1. It should be noted that mitoxantrone, with its bis-secondary amine side chains, cannot be derivatised to an N-oxide. Two SV40 transformed fibroblast cell lines (MRC5-V1 and AT5BIVA) were used, the latter cell line expressing elevated levels of topoisomerase II in all phases of the cell cycle, making it a useful hypersensitive indicator cell line for cellular responses to cleavable-complex-trapping agents (e.g. the aminoacridine mAMSA [19]). Our findings support the use of N-oxide alkylaminoanthraquinones as potential prodrugs of topoisomerase-targeting anticancer agents.

Materials and methods

Cell culture and drug sources

The SV40 transformed human fibroblast cell lines MRC5-V1 (normal donor) and AT5BIVA (ataxia telangiectasia homozygote donor) were grown as asynchronous cultures as described previously [19]. Synchronization was achieved by plating of cells followed by incubation for 48 h under normal conditions prior to exposure to 1 μg/ml aphidicolin (Sigma) for 24 h to induce the accumulation of cells in the early S phase. Arrested cultures were released into fresh medium for 2 h prior to drug treatments. Synchronization was verified by flow-cytometric analysis. Mitoxantrone, {1,4-dihydroxy-5,8-bis[(2-[-hydroxyethyl)amino]ethyl]amino)]-9,10-anthracenedione dihydrochloride (Novantrone), was kindly supplied by Dr. A. Man (Lederle Laboratories, Gosport, UK) as a solid and was stored as aqueous stock solutions of 2 m*M* at 4 °C. The analogues (see Fig. 1, Table 1) were synthesised as previously described [11] and were stored at 4 °C as aqueous stock solutions of 2–11 m*M*.

Assay for clonogenic potential

Prior to drug treatment, cells were plated at a density of 250 cells/well in 6-well plates and then incubated overnight to allow attachment. After a 1-h period of incubation with drug, cells were washed twice in phosphate-buffered saline, then incubated in fresh growth medium for 14 days. Colonies were fixed, stained and counted. Survival data were analysed using a linear transform of the multi-target survival curve [23].

Inhibition of DNA topoisomerase II activity and drug-induced DNA-protein cross-linking

Decatenation activity was assayed according to the electrophoretic method of Sahai and Kaplan [17], with 350 m*M* NaCl-extracted nuclear protein preparations from AT5BIVA cells being used as described previously [19] to catalyse the decatenation of a network of kinetoplast DNA in the presence or absence of drug. The K^+-sodium dodecyl sulfate (SDS) precipitation method was used to measure the tight association of protein with DNA and has been described elsewhere [5]. In brief, the method involves the precipitation of radiolabeled cellular DNA cross-linked to protein in lysates of drug-treated cells.

Measurement of DNA synthesis rates

The method has been described elsewhere [5]. In brief, cultures were labeled with 1.85×10^{-4} MBq/ml [^{14}C]-thymidine (sp. act. 1.92 GBq/mmol, Amersham International) for 48 h, which was followed by a 2-h chase period in fresh medium prior to drug treatment. Replicating DNA was labeled by incubation of cells with 0.37 MBq/ml [^{3}H]-thymidine (sp. act. 2.85 TBq/mmol, Amersham International) during the final 15 min of a 1-h drug exposure. Radioactivity in trichloroacetic acid (TCA) precipitates of cells was determined by liquid scintillometry. The relative rate of DNA synthesis was calculated as follows: $100 \times [^{3}\text{H(drug-treated)}/^{14}\text{C(drug-treated)}]/[^{3}\text{H (control)}/^{14}\text{C (control)}]$.

Results

DNA topoisomerase II inhibition

Inhibition of kinetoplast DNA decatenation activity was measured for the various drugs. The catenated DNA substrate cannot migrate from the well of an agarose gel unless it has previously been exposed to the catalytic action of topoisomerase II, resulting in the release of DNA circles from the catenated complex. Preliminary experiments confirmed the migration properties of kinetoplast preparations and the adenosine triphosphate (ATP) dependence of decatenation. When gel electrophoresis was carried out in the presence of ethidium bromide there was a loss of the fluorescence of DNA in the wells at high concentrations (>20 μ*M*) of mitoxantrone, AQ4 and AQ6, which was also observed to a reduced degree with AQ6NO but not with AQ4NO (data not shown). Loss of fluorescence is indicative of displacement of ethidium bromide from DNA by residual drug binding. To abrogate ethidium displacement, gels were run in the presence of 0.1% SDS and stained after electrophoresis with ethidium bromide.

Figure 2 shows a representative assay in which the release of kinetoplast DNA from the well by the nuclear extract alone gives rise to species of monomers with different supercoiling states (topoisomers). Mitoxantrone and AQ6 inhibited release from the well, revealed a background of nicked monomers and produced rapidly migrating supercoiled intermediates. Inhibition by AQ6NO was detectable. Figure 2 also illustrates the effect of adding drug after the decatenation reaction had been completed (+), showing that the fluorescence intensity and migration patterns of the released DNA circles were not affected by the presence of the drug. In a parallel study [13] the various drugs were examined for their in vitro capacity to bind to calf-thymus DNA as determined by spectrophotometric titration of drug-DNA mixtures. In order of affinity constants the agents ranked as follows: mitoxantrone $(6.19 \times 10^{6}\ M^{-1}) >$ AQ6 $(5.50 \times 10^{6}\ M^{-1}) >$ AQ4 $(3.21 \times 10^{6}\ M^{-1}) >$ AQ6NO $(1.29 \times 10^{6}\ M^{-1}) >$ AQ4NO (DNA binding not detected). We observed the same ranking for ethidium displacement in decatenation assays when ethidium was present during electrophoresis (data not shown).

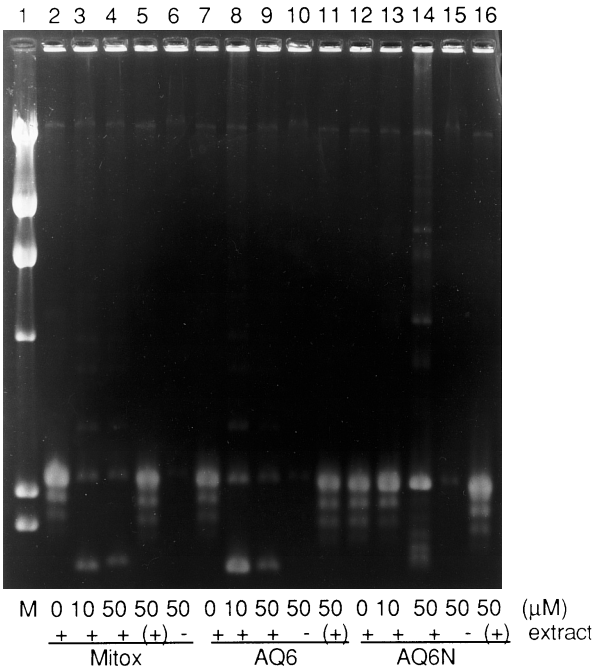

Fig. 2 Representative assay showing dose-dependent inhibition of the topoisomerase II-decatenation activity of a nuclear protein extract by mitoxantrone (*Mitox*), AQ6 and AQ6NO (*AQ6N*) (*M* Molecular-weight markers/*Hin* dIII digest of λ DNA representing 23.13, 9.42, 6.56, 4.36, 2.32 and 2.03 kb; *extract* nuclear protein extract containing topoisomerase II; (+) drug was added at the end of the incubation period). Samples were incubated for 90 min at 37 °C in the presence or absence of drug

The results of the assays for inhibition of the catalytic activity of topoisomerase II from nuclear extracts indicated that mitoxantrone had activity similar to that of AQ6, with AQ4 requiring approximately a doubling of the drug concentration to effect the same level of inhibition. AQ6NO required at least a 5-fold greater concentration than did the parental agent AQ6 to effect inhibition, whereas AQ4NO was inactive for concentrations up to 80 μ*M*. We conclude that the relative abililty of the agents to inhibit topoisomerase II in a cell-free system reflects their DNA-binding capacity.

Drug-induced DNA-protein cross-linking

Topoisomerase II trapping, measured indirectly using the K^{+}-SDS precipitation assay, was evaluated for each derivative using intact cells with low (MRC5-V1) and elevated (AT5BIVA) levels of topoisomerase II (Fig. 3). Cross-linking by mitoxantrone became saturated at doses above 5 μ*M* but was clearly dependent upon enzyme availability. In MRC5-V1 cells, AQ6 showed activity similar to that of mitoxantrone, whereas AQ4 showed reduced activity. In AT5BIVA cells the

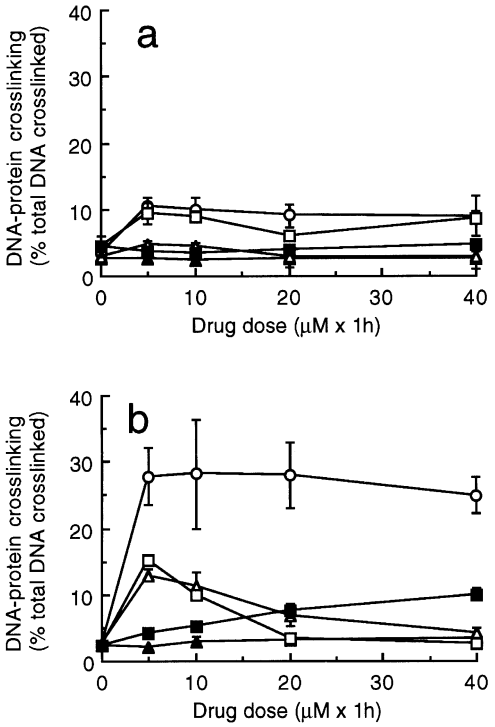

Fig. 3a, b Dose-dependent induction of DNA-protein cross-linking in human cells assayed immediately following 1 h exposure to anthraquinones. **a** MRC5-V1. **b** AT5BIVA. (○ Mitoxantrone, △ AQ4, □ AQ6, ▲ AQ4NO, ■ AQ6NO) Data represent mean values derived from 3 experiments (±SE)

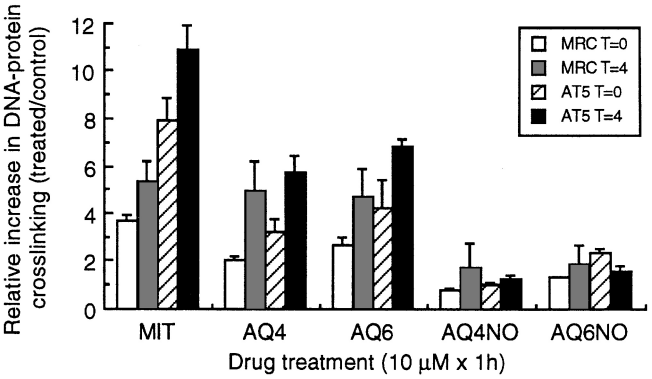

Fig. 4 Persistence of drug-induced DNA-protein cross-links in cells exposed for 1 h to anthraquinones. Cross-linking was determined either immediately after drug treatment ($T = 0$) or after a 4-h recovery period ($T = 4$) in drug-free medium. Data represent mean values derived from 3 experiments (±SE)

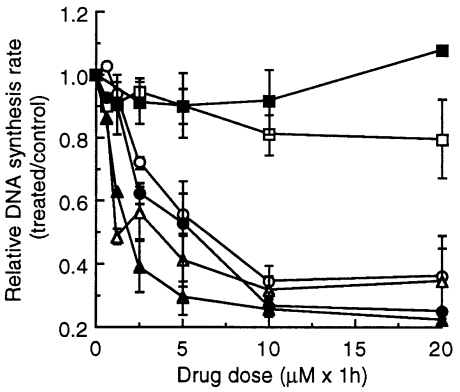

Fig. 5 Inhibition of DNA synthesis in S-phase-synchronized cultures as measured at the end of a 1-h exposure to either mitoxantrone (*circles*), AQ6 (*triangles*) or AQ6NO (*squares*). Drugs were added to cultures at 2 h after release from aphidicolin block. Details of APC synchronisation are given in Materials and methods. (○, △, □ MRC5-V1; ●, ▲, ■ AT5BIVA)

increased availability of topoisomerase II differentiated between high levels of cross-linking with mitoxantrone and reduced but similar cross-linking with AQ4 and AQ6. Cross-linking by AQ4 and AQ6 showed evidence of inhibition at high drug concentrations. The AQ4NO derivative was essentially inactive. In AT5BIVA cells, AQ6NO ($\geq 20\ \mu M$) showed significant activity as reflected by a >8-fold difference in the concentration of drug required to induce equivalent cross-linking as compared with AQ6. Thus, the general patterns observed were similar to those obtained in the cell-free system, namely (1) activity of mitoxantrone, AQ6 and AQ4; (2) significantly reduced but detectable activity of AQ6NO; and (3) inactivity of AQ4NO. Figure 4 shows that the DNA-protein cross-links induced by mitoxantrone or the analogues tended to persist or even accumulate during post-treatment incubation.

DNA synthesis inhibition

Figure 5 shows that there was no significant cell-line-dependent difference in the initial capacity of three selected anthraquinones to inhibit DNA synthesis. Inhibition in S-phase-synchronized cultures was biphasic for mitoxantrone and AQ6, the former showing a dose dependence that correlated with the level of cross-linking, whereas AQ6NO appeared to be relatively inactive. The results do not support a simple relationship between enzyme trapping and DNA synthesis inhibition, given the consistently enhanced activity of AQ6 (1.25–5 μM, Fig. 5) and the similarity of the cell-line responses.

Cytotoxicity studies

Representative survival curves are shown in Fig. 6, and the combined survival data are shown in Table 2 together with values for other topoisomerase II poisons. Considering MRC5-V1 cells first, D_0 values (the dose required to reduce survival by 37% as measured on the linear part of the survival curve) of 27.3 nM for mitoxantrone and 49.0 nM for AQ4 were obtained. Unexpectedly, given the results of the above-mentioned

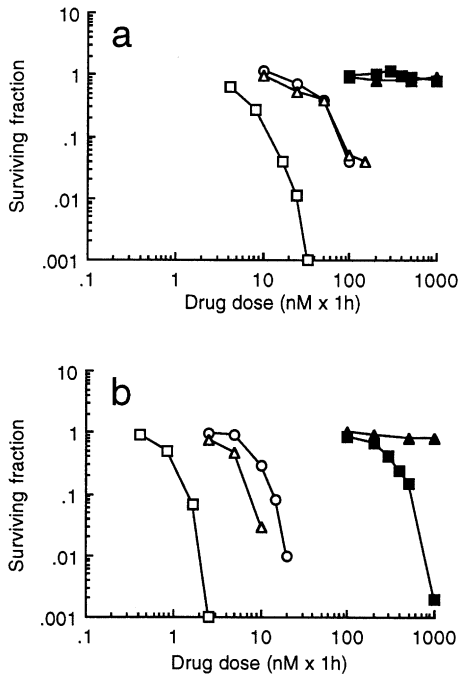

Fig. 6a, b Cytotoxicity of 1 h exposure to anthraquinones as determined by clonogenic potential. **a** MRC5-V1. **b** AT5BIVA. (○ Mitoxantrone, △ AQ4, □ AQ6, ▲ AQ4NO, ■ AQ6NO) Representative survival curves are shown (mean values for quadruplicate determinations, SD ≤ 10%)

studies, AQ6 (D_0 value 4.8 n*M*) was found to be more cytotoxic than either mitoxantrone or AQ4. D_0 values for AQ4NO and AQ6NO were estimated to be > 20 and > 1.4 μ*M*, respectively, indicating at least a 50-fold reduction in the toxicity of the N-oxides, with AQ6NO showing a potency similar to that of aminoacridine mAMSA (Table 2). AT5BIVA cells were 7, 21 and 14 times more sensitive than MRC5-V1 cells to mitoxantrone, AQ4 and AQ6, respectively (Table 2). Although AQ6NO showed a significant cytotoxic effect (D_0 value 147 n*M*) towards AT5BIVA cells in the dose range used, AQ4NO showed low cytotoxicity (D_0 value 18.5 μ*M* as estimated by survival curve fitting).

Discussion

This report relates the structures of novel anthraquinones to one of the possible initiating events for cytotoxicity, namely topoisomerase inhibition. The in vitro capacity of the alkylaminoanthraqauinones to bind calf-thymus DNA [13] reflected their in vitro capacity to inhibit kinetoplast DNA decatenation. The results are consistent with a requirement of DNA binding for efficient enzyme trapping by the analogues. In intact cell systems a similar general ranking was observed for drug-induced DNA-protein cross-linking, an indirect measure of topoisomerase II trapping, although the cross-linking activity of AQ4 and AQ6 appeared to be reduced at high concentration (> 10 μ*M*). Under conditions of overexpression of topoisomerase II the cytotoxic potential of AQ4, AQ6, AQ6NO and mitoxantrone was enhanced. The exception was AQ4NO, which was essentially inactive. The results confirm a requirement of DNA binding for efficient enzyme inhibition. The topoisomerase studies were carried out as supralethal drug concentrations, and it is possible that differences in the sequestration or availability of free drug in cells may affect the dynamics of interaction with the enzyme. Indeed, we present evidence that cross-linking continued to occur in cells released from drug treatment. However, the hypersensitivity of the topoisomerase II overproducer to the alkylaminoanthraquinones strongly implicates topoisomerase II-dependent DNA cleavage as an important factor in the cytotoxicity responses within the biological dose range.

Table 2 Cytotoxicity data for anthraquinones and other DNA topoisomerase II poisons (*Mitox* Mitoxantrone, *ND* not determined, D_0 dose required to reduce survival by 37% as measured on the linear part of the survival curve, *D50* dose required to reduce survival by 50%, *n* extrapolation number for the linear portion of the survival curve; see Watson [23])

Compound	Survival curve parameters[a]						Relative cytotoxicity (D_0 MRC5-V1/D_0 AT5BIVA)	D_0 drug/D_0 Mitox (for MRC5-VI cells)
	MRC5-V1 cells			AT5BIVA				
	D_0[b]	D50[b]	*n*	D_0[b]	D50[b]	*n*		
VP-16	7000							256
m-AMSA	2900							106
Doxorubicin	500							18.3
Mitoxantrone	27.3	38	1.6	3.8	7.3	2.2	7.2	Set at 1.0
AQ4	49.0	30	1.0	2.3	4.6	3.7	21.3	1.8
AQ4NO	>1000	>1000		>1000	>1000		ND	>730
AQ6	4.8	5.1	1.3	0.34	0.81	3.0	14.1	0.18
AQ6NO	1390	>1000	1.5	146.5	255	2.9	9.5	51

[a] Values derived from the combined data from 3–4 experiments
[b] Units of n*M* for a 1-h exposure

The N-oxide derivatives can be described formally as R_3N^+-O^- yielding electrically neutral side chains and agents that are significantly less basic. Cationic charge and basicity are important physicochemical properties influencing the transport and macromolecule binding of drugs. In parallel studies we have employed flow cytometry, and confocal imaging [21] to investigate the uptake and subcellular distribution of these agents in intact cells (Smith and Blunt, unpublished observations). N-oxide substitution on one side chain diminished nuclear accumulation, whereas substitution on both side chains abolished it almost completely. No significant difference between the two cell lines in whole-cell or nuclear uptake of alkylaminoanthraquinones was detectable by fluorometry. However, AQ6 nuclear targeting was enhanced as compared with that noted for mitoxantrone. Thus, under low-dose conditions, within the biological range, preferential uptake of AQ6 as compared with the other agents may explain its high level of relative cytotoxicity.

DNA binding and presumably related nuclear targeting, but not topoisomerase II trapping per se, appear to be important features for the initial inhibition of DNA synthesis. The retention of some topoisomerase-II-targeting capacity in both cell-free and intact cell systems by AQ6NO is presumably a reflection of the residual DNA-binding activity contributed by the $H_2N(CH_2)_2NH(CH_2)_2OH$ side chain. In the case of AQ6NO the greater residual cytotoxic and DNA/nuclear targeting activities would serve to increase basal activity towards proliferating cells while offering a high level of differential toxicity in hypoxic cells, should the N-oxide be bioreduced to AQ6, its putative metabolite. Thus, the persistent nature of anthraquinones and the longevity of topoisomerase-associated DNA damage described herein and elsewhere [5] should provide activity towards such cells attempting to re-enter the cell cycle.

This study supports the concept of the N-oxides as prodrugs of topoisomerase-targeting alkylaminoanthraquinones. For AQ6NO this is of interest because it retains activity against cells with appropriate presentation of topoisomerase II, whereas its putative metabolite AQ6 is up to 400 times more cytotoxic. Using a hypersensitive cell line, we have shown that AQ6NO clearly traps topoisomerase II in intact cells. Thus, the mono-N-oxide would show some activity against rapidly proliferating cells with generally elevated topoisomerase IIα levels [6] while retaining the potential for bioreductive conversion in hypoxic cells to a highly active product (AQ6). Indeed, the latter compound has an even greater in vitro cytotoxicity than does mitoxantrone. As a di-N-oxide, AQ4NO is effectively inactive as a cytotoxic agent but has the potential for conversion to a metabolite, AQ4, which has a cytotoxic potency similar to that of mitoxantrone. In support of the bioreductive potential of these N-oxides, AQ4NO has been shown to undergo quantitative conversion to AQ4 under anaerobic conditions in biological systems [12] and shows positive interaction with radiation in vivo [8]. AQ4NO and AQ6NO are currently undergoing further pre-clinical trials in vivo to establish their potential as bioreductive agents.

References

1. Bowden GT, Roberts R, Alberts DS, Peng Y-M, Garcia D (1985) Comparative molecular pharmacology in leukaemic L1210 cells of the anthracene anticancer drugs mitoxantrone and bisantrene. Cancer Res 45:4915
2. Brown JM, Giaccia AJ (1994) Tumour hypoxia: the picture has changed in the 1990s. Int J Radiat Biol 65:95
3. Cress AE, Roberts RA, Bowden GT, Dalton WS (1988) Modification of keratin by the chemotherapeutic drug mitoxantrone. Biochem Pharmacol 37:3043
4. Drake FH, Hofmann GA, Bartus HF, Mattern MR, Crooke ST, Mirabelli CK (1989) Biochemical and pharmacological properties of p170 and p180 forms of topoisomerase II. Biochemistry 28:8154
5. Fox ME, Smith PJ (1990) Long-term inhibition of DNA synthesis and the persistence of trapped topoisomerase II complexes in determining the toxicity of the antitumour DNA intercalators mAMSA and mitoxantrone. Cancer Res 50:5813
6. Heck MM, Hittelman WN, Earnshaw WC (1988) Differential expression of DNA topoisomerases I and II during the eukaryotic cell cycle. Proc Natl Acad Sci USA 85:1086
7. Liu LF (1989) DNA topoisomerase poisons as anti-tumour drugs. Annu Rev Biochem 58:351
8. Mckeown SR, Hejmadi MV, McIntyre IA, McAleer JJA, Patterson LH (1995) AQ4N: an alkylaminoanthraquinone N-oxide showing bioreductive potential and positive interaction with radiation in vivo. Br J Cancer 72:76
9. Overgaard J (1989) Sensitization of hypoxic tumour cells – clinical experience. Int J Radiat Biol 56:801
10. Parliament MB, Chapman JD, Urtasun RC, McEwan AJ, Golberg L, Mercer JR, Mannan RH, Wiebe LI (1992) Non-invasive asessment of human tumour hypoxia with ^{123}I-iodazomycin arabinoside: preliminary report of a clinical study. Br J Cancer 65:90
11. Patterson LH (1989) Anthraquinone anticancer compounds with (disubstituted amino-N-oxide)alkylamino substituent. UK Patent GB 2 237 283. UK Patent Bureau, London
12. Patterson LH (1993) Rationale for the use of aliphatic N-oxides of cytotoxic anthraquinones as prodrug DNA binding agents: a new class of bioreductive agent. Cancer Metastasis Rev 12:119
13. Patterson LH, Maine JE, Cairns DC, Craven MR, Bennett N, Fisher GR, Ruparelia K, Giles Y (1992) N-oxides of DNA affinic agents as bioreductively activated prodrugs. Proc Am Soc Cancer Res 33:2571
14. Patterson LH, Craven MR, Fisher GR (1994) Aliphatic amine N-oxides of DNA binding agents as bioreductive drugs. Oncol Res 6:533
15. Roberts RA, Cress AE, Dalton WS (1989) Persistent intracellular binding of mitoxantrone in a human colon carcinoma cell line. Biochem Pharmacol 38:4283
16. Rowe TC, Chen GL, Hsiang Y-H, Liu LF (1986) DNA damage by antitumour acridines mediated by mammalian DNA topoisomerase II. Cancer Res 46:2021
17. Sahai BM, Kaplan JG (1986) A quantitative decatenation assay for type II topoisomerases. Anal Biochem 156:134
18. Shenkenberg TD, Von Hoff DD (1986) Mitoxantrone: a new anti-cancer drug with significant clinical activity. Ann Intern Med 105:67

19. Smith PJ, Makinson TA (1989) Cellular consequences of overproduction of DNA topoisomerase II in an ataxia-telangiectasia cell line. Cancer Res 49 : 1118
20. Smith PJ, Morgan SA, Fox ME, Watson JV (1990) Mitoxantrone-DNA binding and the induction of topoisomerase II associated DNA damage in multi-drug resistant small cell lung cancer cells. Biochem Pharmacol 40 : 2069
21. Smith PJ, Sykes HR, Fox ME, Furlong IJ (1992) Subcellular distribution of the anticancer drug mitoxantrone in human and drug-resistant murine cells analyzed by flow cytometry and confocal microscopy and its relationship to the induction of DNA damage. Cancer Res 52 : 1
22. Wang JC (1987) Recent studies of DNA topoisomerases. Biochim Biophys Acta 909 : 1
23. Watson JV (1978) A linear transform of the multi-target survival curve. Br J Radiol 51 : 534