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ORIGINAL ARTICLE

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Carbamate analogues of amsacrine active against non-cycling cells: relative activity against topoisomerases IIα and β

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Abstract *Purpose*: Methyl N-(4′-(9-acridinylamino)-phenyl)carbamate hydrochloride (AMCA) and methyl N-(4′-(9-acridinylamino)-2-methoxyphenyl)carbamate hydrochloride (mAMCA) are analogues of the topoisomerase II (topo II) poison amsacrine, and are distinguished from amsacrine by their high cytotoxicity towards non-cycling cells. Since mammalian cells contain two forms (α and β) of topo II and the α isoform is down-regulated in non-cycling cells, we have considered whether these carbamate analogues target topo IIβ selectively. *Methods*: A drug permeable yeast strain (JN394 *top2-4*) was transformed using a shuttle vector containing either human *top2α*, human *top2α* or yeast *top2* under the control of a GAL1 promoter. The strain was analysed at a non-permissive temperature, where only the plasmid-borne topo II was active. *Results*: AMCA and mAMCA produced comparable levels of cell killing with human DNA topo IIα, human DNA topo IIβ and yeast DNA topo II. Two other acridine derivatives N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) and its 7-chloro derivative, which like AMCA and mAMCA are able to overcome multidrug resistance mechanisms, were much more active against human DNA topo IIα than against human DNA topo IIβ and yeast DNA topo II. A series of mutant Chinese hamster and human lines with defined topo lesions, including the HL60/MX2 line that lacks topo IIβ expression, was also used to compare resistance to amsacrine, AMCA and etoposide. Loss of topo IIβ activity had a greater effect on amsacrine and AMCA than on etoposide. Resistance of murine Lewis lung cultures in exponential and plateau phase was also measured. Loss of topo IIα activity, as measured in both mutant cells expressing lower amounts of enzyme and in cells in plateau phase, resulted in concomitant acquisition of resistance that was greatest for etoposide and least for AMCA. *Conclusion*: We conclude that the carbamate analogues of amsacrine recognize both topo IIα and β in cells.

Key words DNA topoisomerase II · AMCA · mAMCA · DACA · Cl-DACA

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Introduction

Type II DNA topoisomerases are essential cellular enzymes that alter DNA topology by passing one DNA helix through a transient, enzyme-bridged, double-stranded DNA break in another [2, 28, 29, 34]. DNA topoisomerase II (topo II) is a target for a number of clinically important antitumour agents including etoposide and amsacrine. Such anti-topo II agents exert their cytotoxicity by stabilizing an enzyme-DNA intermediate, termed a "cleavable complex", in which the

two cleaved DNA strands are covalently linked at their 5′-terminal phosphates to conserved tyrosine residues in the enzyme, one DNA strand to each monomer. The cleavable complexes are processed by the cell in a cascade of events, possibly involving the replication fork and DNA helicase [21], which lead to permanent double-stranded DNA breaks and eventually, cell death [9].

Human and other mammalian cells produce two genetically distinct topo II isoforms, α and β, unlike lower eukaryotes such as *Drosophila melanogaster* and *Saccharomyces cerevisiae*, which appear to have only a single enzyme form. The two human isoforms vary in their cell cycle expression and cellular localization, with topo IIα regulated in a cell cycle–dependent manner and topo IIβ regulation less cell cycle dependent. This suggests different roles within the cell and so potentially two distinct targets in cancer chemotherapy.

The acridine derivative amsacrine [5] targets topo II [25] and is used clinically in the treatment of acute leukaemia [37]. During development of amsacrine and its analogues, two further compounds were produced (see Fig. 1 for structures), methyl N-(4′-(9-acridinylamino)-phenyl) carbamate hydrochloride (AMCA) and methyl N-(4′-(9-acridinylamino)-2-methoxyphenyl) carbamate hydrochloride (mAMCA) [6]. These appear to target topo II, as shown by the induction of DNA breaks by purified topo IIα and β [4], but are distinguished from amsacrine by their high cytotoxicity towards non-cycling cells [16]. This has led to the suggestion that they may be preferentially targeting DNA topo IIβ, the predominant isoform in plateau phase cells [2, 4].

Multidrug resistance (MDR) is a major problem in the treatment of cancer and the potential of some of these acridine derivatives to overcome this resistance is very encouraging. AMCA and mAMCA are both able to overcome transport-related MDR in cell lines [15] and their cytotoxicity in cycling cells is not altered by co-incubation with aphidicolin, an inhibitor of DNA polymerase, or by PD128763, an inhibitor of poly-(ADP-ribose) polymerase associated with the replicative complex [24]. More recently, a new series of acridine derivatives with high antitumour activity has been synthesized [1]. These include N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA), which is currently completing Phase I clinical trials, and its 7-chloro derivative, Cl-DACA (see Fig. 1 for structures). DACA induces protein-DNA cross-links in L1210 cells [31] and overcomes multiple MDR mechanisms [10, 17]. Both DACA and Cl-DACA stimulate DNA cleavage in response to topo I as well as targeting topo II [18].

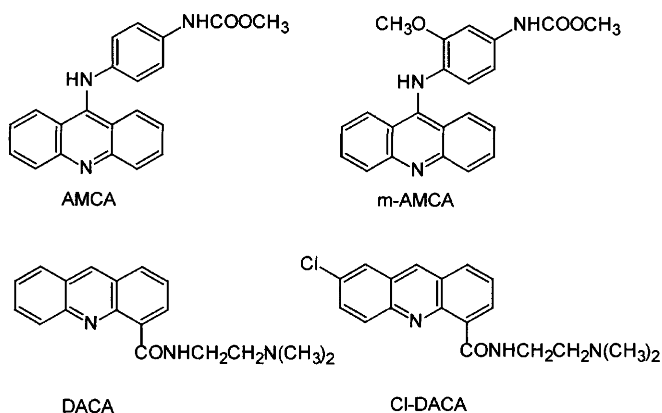

Fig. 1 Structures of the acridine derivatives used in this study

The analysis of the interaction of drugs with topo II has been greatly facilitated by the use of yeast model systems. Nitiss and Wang developed a system that has been used extensively to analyse the effects of anticancer agents on yeast *Saccharomyces cerevisiae* [27]. While such an approach has provided a wealth of information about drug interactions with yeast topo II, the more relevant targets for cancer chemotherapy are human topo IIα and β. We have previously reported the development of this heterologous yeast system to study human topo IIα [35] and topo IIβ [3, 22]. In this paper we have used these systems to analyse the effects of AMCA and mAMCA on topo IIα and β, comparing their action with those of DACA and Cl-DACA, and with previously reported data for other topo poisons [3, 35]. In addition, we have investigated the action of AMCA in a range of human and Chinese hamster ovary (CHO) cell lines with known defects in topo II expression, as well as Lewis lung carcinoma (LLTC) cells expressing different cellular amounts of topo IIα. We have compared the results of these compounds with those of other topo II poisons.

Materials and methods

Materials

Drugs were synthesized in the Auckland Cancer Society Research Centre [6, 11]. For the yeast experiments, they were dissolved in dimethylsulfoxide (DMSO). Recombinant human topo IIα and β were purified as described previously [3, 35]. All other reagents were obtained from commercial sources. The *S. cerevisiae* strain JN394t2-4 *Matα ISE2 ura3-52 top2-4 rad52::LEU2* was kindly provided by Professor J.C. Wang (Harvard University) [27]. The plasmids YEpWOB6, YEphTOP2βKLM and YEpTOP2-PGAL1(G1T2) have been described previously [3, 22, 35].

Wild-type and etoposide-resistant (V6) human small cell lung cancer cell lines (H209) were kindly provided by Dr. Susan P.C. Cole (Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada) [13]. Wild-type and mitoxantrone-resistant (MX-2) HL60 cell lines were a generous gift from Dr. W. Graydon Harker (Veterans Affairs Medical Center and Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah, USA).

Methods

Cleavage of plasmid DNA

Cleavage reactions were carried out in 50 m*M* Tris-HCl buffer, pH 7.5, 100 m*M* KCl, 10 m*M* $MgCl_2$, 0.1 m*M* DTT, 0.5 m*M* EDTA, 30 μg/ml BSA, 500 ng supercoiled pBR322 and 200 ng of human topo IIα or β, in the presence or absence of the drugs. After incubation for 30 min at 37 °C, SDS was added to a final concentration of 1% followed by addition of proteinase K to a final

concentration of 0.5 mg/ml and incubation continued for 30 min at 50 °C. Loading buffer was added (0.5% SDS, 25% glycerol, 0.1% bromophenol blue) and samples were analysed by electrophoresis in 0.8% agarose in TBE buffer (89 m*M* Tris, 89 m*M* boric acid and 2.5 m*M* EDTA). Gels were stained with ethidium bromide and photographed under UV transillumination.

Inhibition of relaxation

Inhibition of relaxation reactions were carried out in 50 m*M* Tris-HCl buffer, pH 7.5, 100 m*M* KCl, 10 m*M* $MgCl_2$, 0.1 m*M* DTT, 0.5 m*M* EDTA, 30 μg/ml BSA, 500 ng supercoiled pBR322 and 200 ng of human topo IIα or β, in the presence or absence of the drugs. After incubation for 30 min at 37 °C, loading buffer was added (0.5% SDS, 25% glycerol, 0.1% bromophenol blue) and samples were analysed by electrophoresis in 0.8% agarose in TBE buffer (89 m*M* Tris, 89 m*M* boric acid and 2.5 m*M* EDTA). Gels were stained with ethidium bromide and photographed under UV transillumination. The amount of cleavage was determined by scanning the gels using a Biorad documentation system and Molecular Analyst software. Quantification of the linearised form of DNA was carried out using TINA version 2.08e software.

Determination of drug sensitivity in an in vivo yeast system

Drug sensitivity was analysed using a short-term drug exposure in liquid culture followed by growth in drug-free plates [22]. Briefly, logarithmically growing yeast cells (strain JN394t2-4 transformed with a human *TOP2α*, human *TOP2β* or *S. cerevisiae TOP2* plasmid) were cultured in selective medium lacking uracil, at 25 °C, diluted to a titre of 5×10^6 cells/ml in complete medium (YPDA) containing yeast extract, bacto-peptone, dextrose and adenine sulphate. They were then grown for 1 h at 35 °C to inactivate the yeast topo II derived from the host *top2-4* allele, so the only active topo II was produced by the plasmid-borne *TOP2* gene. Drug or solvent was added and growth was continued for a further 16 h at 35 °C. The yeast were diluted appropriately, plated in triplicate in YPDA agar (48 °C) without drug, incubated for 3–4 days at 35 °C, and the number of colonies counted. The percentage survival was determined by comparing the number of colonies in the no-drug control culture to those on the drug-treated culture. The IC_{50} was defined as the drug concentration reducing the number of colonies by 50% compared with cells grown in the absence of drug. All experiments were repeated at least three times and the means and population standard deviations were calculated for each set of data. The statistical significance of the differences between dose-response curves was determined using Student's *t*-test (2 tailed paired).

Mammalian cell culture

All cell lines were grown in a humidified 5% CO_2 atmosphere. The derivation of CHO mutant cell lines $VPMR_5$, MTZ_4, SMR_5 and SMR_{16} has been described [19, 32, 33, 36]. All CHO cell lines were grown in α-modified minimal essential medium supplemented with 5% foetal bovine serum (FBS). Cytotoxicity in CHO cell lines was assessed by clonogenic assay following 4-h drug exposures [36]. Wild-type and etoposide-resistant (V6) human small cell lung cancer (H209) cell lines were cultured in RPMI 1640 medium supplemented with 5% FBS and 4 m*M* glutamine. Wild-type and mitoxantrone-resistant (MX-2) HL60 cell lines were grown in RPMI 1640 medium supplemented with 10% FBS and 2 m*M* glutamine. Cytotoxicity in H209 and HL60 cell lines was assessed by MTT assay following a 72-h drug exposure [8]. Comparisons of etoposide, amsacrine and AMCA were made in a single experiment utilizing at least three cell lines (including the wild-type line) to ensure relevant comparison. Composite survival curves were constructed from the mean of between two and five separate experiments, from which individual IC_{50} values were read for comparative purposes. To allow direct comparison of the different cell lines, resistance factors were calculated by dividing the IC_{50} of the cell line with a particular drug by the IC_{50} of the wild-type cell line with the same drug.

Western blotting

LLTC cells were grown in exponential or to plateau phase as previously described [16]. Cells were centrifuged (1500 *g*, 4 °C), the supernatant was aspirated to leave 50 μl of medium, and the cells were resuspended. Cell suspensions were added, with mixing, directly to wells of a 6% SDS-PAGE gel containing 50 μl of 2 × strength sample loading buffer, thus allowing rapid lysis of cells. After electrophoresis, the protein bands were electroblotted on to Immobilon-P membrane (Millipore, Bedford, Mass., USA). Membranes were probed in Tris-buffered saline, pH 7.5, containing 2% BSA and 1% (v/v) Tween 20, using antibodies to topo II (TopoGEN). Bands were resolved using goat anti-rabbit secondary antibodies (Vectastain Elite, Vector Laboratories, Burlingame, Calif., USA) and enhanced chemiluminescence (Amersham Life Science, Little Chalfont, UK).

Results

Comparison of human DNA topo IIα and β interactions with AMCA, mAMCA, DACA, and Cl-DACA using purified enzymes

The effects of the acridine derivatives AMCA, mAMCA, DACA and Cl-DACA on purified recombinant human topo IIα and human topo IIβ were analysed by promotion of cleavage of supercoiled pBR322 DNA. AMCA, mAMCA and DACA all promoted cleavage of supercoiled plasmid DNA while Cl-DACA did not promote topo II cleavage, in agreement with previous studies [4, 18]. The amount of cleavage by both isoforms at a range of drug concentrations was determined and levels of cleavage by the two topo II isoforms were found to be exactly the same at lower drug concentrations, with maximum cleavage observed at 5 μ*M* for all three drugs. Once the maximum level of cleavage was reached, the amount of cleavage by topo IIβ seemed to decrease at a faster rate than that of topo IIα (Fig. 2A–C). The ability of the four acridines to inhibit the relaxation activity of the purified recombinant proteins was also investigated. The isoforms were found to be inhibited in their relaxation activity to equivalent amounts for all four of the compounds tested at a drug concentration of 1 μ*M*.

Drug sensitivity of *S. cerevisiae* strain JN394top2-4 expressing human DNA topo IIα or β, or *S. cerevisiae* topo II

The yeast strain JN394t2-4 *Matα ISE2 ura3-52 top2-4 rad52::LEU2* [27] has the *top2-4* mutation allowing growth at 25 °C but not 35 °C. The *ISE2* permeability mutation facilitates drug uptake, and the *rad52* mutation renders the yeast defective in double-stranded DNA break repair, thereby sensitising the strain to the effects of topo-targeting agents [26]. In previous work, it has

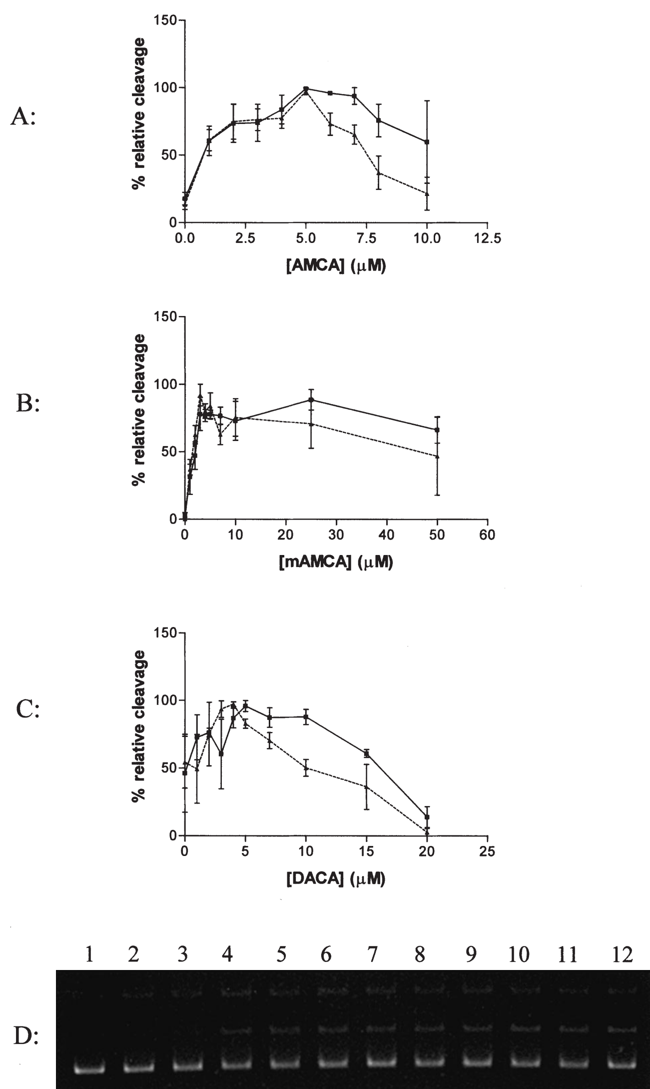

Fig. 2A–C Percentage cleavage of pBR322 DNA by topo IIα (- - -▲- - -) and topo IIβ (—■—) in the presence of the indicated concentrations of AMCA, mAMCA and DACA. Error bars indicate the standard deviation from the mean. **Fig. 2D** A representative agarose gel showing the cleavage of supercoiled pBR322 in the presence of topo IIα and AMCA. *Lane 1* supercoiled pBR322 alone, *lane 2* pBR322 and topo IIα, *lane 3* (solvent control) pBR322, topo IIα and DMSO, *lane 4* pBR322, topo IIα and 1 μM AMCA in DMSO, *lanes 5–12* as for lane 4 but with AMCA increasing in 1 μM steps to 10 μM in lane 12. *AMCA* methyl N-(4′-9-acridinylamino)-phenyl)carbamate hydrochloride, *mAMCA* methyl N-(4′-9-acridinylamino)-2-methoxyphenyl)carbamate hydrochloride, *DACA* N-[2-(dimethylamino)ethyl]acridine-4-carboxamide, *DMSO* dimethyl sulfoxide, *topo* II topoisomerase II

been shown that growth of JN394t2-4 at 35 °C is strictly dependent on transformation with a plasmid-borne *TOP2* gene [27]. The sensitivity of human DNA topo IIα, β or *S. cerevisiae* DNA topo II to AMCA, mAMCA, DACA and Cl-DACA was tested in JN394t2-4 transformed with YEpWOB6, YEphTOP2βKLM or YEpTOP2-PGAL1(G1T2).

For quantitative analysis of drug sensitivity, short term drug exposure to drug in liquid culture was followed by growth in drug-free agar for 3–4 days [26]. Yeast cells were grown at 25 °C until they reached mid-log phase, diluted and then incubated at 35 °C to inactivate the temperature sensitive host topo II. Either drug or solvent was then added and the cells were grown for a further 16 h at 35 °C. The yeast cells were then diluted appropriately, plated in drug-free YPDA agar and incubated at 35 °C. Individual colonies were counted and the percentage survival calculated by comparison with the cell number in the solvent control cultures. The survival curves were determined for AMCA, mAMCA, DACA and Cl-DACA and are shown in Fig. 3. The IC_{50} values were also determined and are shown, in comparison with amsacrine as a reference compound, in Table 1. The drugs formed two categories: compounds to which the *TOP2α* and *TOP2β* yeast transformants showed similar sensitivity (AMCA, mAMCA and amsacrine), and those to which the *TOP2α* transformant was more sensitive than that bearing *TOP2β* (DACA and Cl-DACA).

Of the four acridines tested, mAMCA exhibited the most dramatic cell killing, exhibiting similar levels of cytotoxicity towards all three transformants. At 20 μg/ml (54 μ*M*) there was no survival of yeast bearing any of the three plasmids. Yeast expressing human topo IIα exhibited 50% survival at 1.3 μg/ml (3.5 μ*M*) mAMCA, whilst yeast expressing human topo IIβ or yeast topo II both showed 50% survival at 1.5 μg/ml (4 μ*M*) mAMCA. There are no significant differences in cytotoxicity to mAMCA between the three transformants. The second carbamate analogue, AMCA, was the next most cytotoxic of the compounds tested.

DACA and Cl-DACA were tested in a concentration range of 5–50 μg/ml (14–137 μ*M* and 12.5–125 μ*M* respectively). For both DACA and Cl-DACA, yeast transformants expressing human topo IIα were the most sensitive of the three transformants investigated. Both DACA and Cl-DACA were less toxic than mAMCA or AMCA with IC_{50} values for topo IIα of 20 μg/ml (55 μ*M*) and 36 μg/ml (90 μ*M*) respectively. Previously, DACA was shown to inhibit its own cytotoxicity [14] and this observation is confirmed by our results. The yeast carrying the plasmid bearing the yeast *TOP2* gene appears to be slightly affected by the drug, with survival dropping to around 65% at 25 μg/ml (68 μ*M*). Yet at the higher DACA concentrations there seems to be a modest recovery, with survival increasing again to 80% at 50 μg/ml (137 μ*M*). Similar cytotoxicity profiles were observed when plateau phase yeast were exposed to plates containing these cytotoxic agents.

Effects of AMCA, amsacrine and etoposide on mammalian cell lines

The carbamate analogues of amsacrine, AMCA and mAMCA retain much of their cytotoxicity against cells in plateau phase [16]. Cultures of the murine LLTC cell line were grown in exponential or plateau phase as pre-

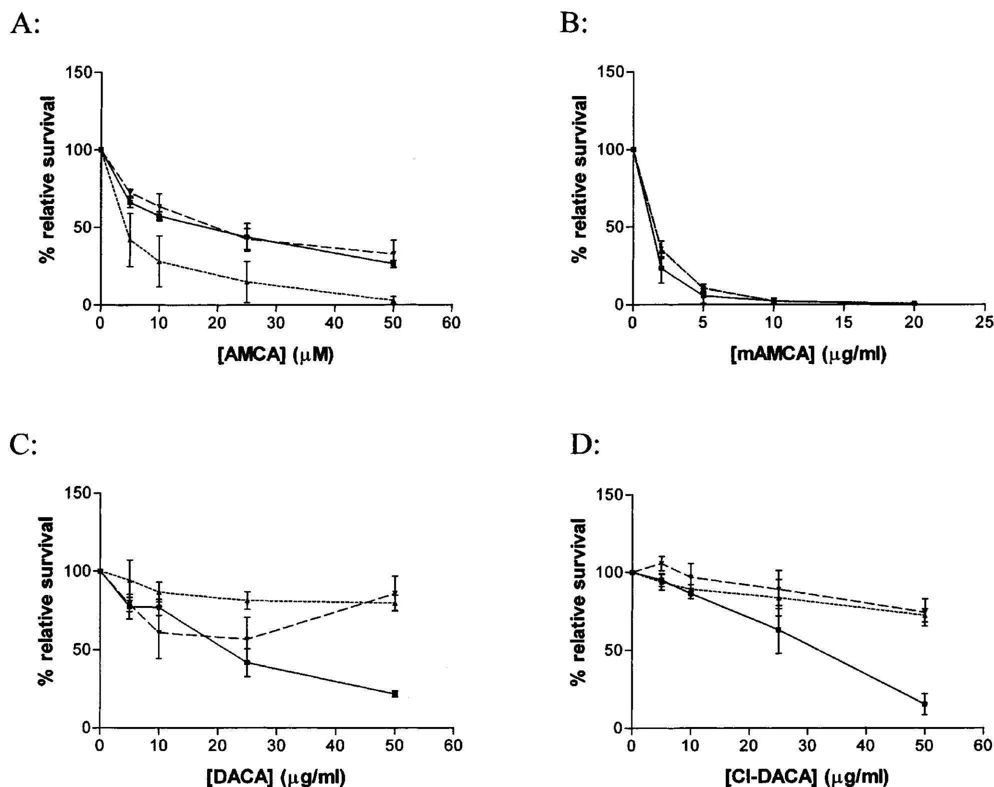

Fig. 3 Viability of yeast JN394*t2-4* transformants expressing human DNA topo IIα (- - -▲- - -), human DNA topo IIβ (—■—) or yeast topo II (- - -▼- - -) following drug exposure. **A** AMCA; **B** mAMCA; **C** DACA; **D** Cl-DACA. Each point is the mean of at least three experiments and error bars indicate the standard deviation from the mean. *AMCA* methyl N-(4′-9-acridinylamino)-phenyl)carbamate hydrochloride, *mAMCA* methyl N-(4′-9-acridinylamino)-2-methoxyphenyl)carbamate hydrochloride, *DACA* N-[2-(dimethylamino)ethyl]acridine-4-carboxamide, *Cl-DACA* 7-chloro derivative of DACA, *topo* II topoisomerase II

viously described [16]. Lysates of plateau phase cells were prepared and assayed for topo IIα content by Western blotting. Different numbers of cells were lysed on each well of the gel and the intensities of the bands were compared. Lysis of 3×10^6 plateau phase cells produced a band of comparable intensity to that of 5×10^5 exponential phase cells, suggesting that the former had approximately 6% of the topo IIα content of the latter, and confirming that plateau phase cells contain low amounts of topo IIα. This has led to the suggestion that AMCA and mAMCA may be preferentially targeting topo IIβ in vivo, since topo IIβ is the predominant isoform in plateau phase cells. To investigate this we obtained cell line HL60/MX-2, a human cell line selected in mitoxantrone. The HL60/MX-2 line has no detectable topo IIβ, and in addition, one allele of topo IIα appears to be modified to produce a truncated protein with altered subcellular localization [20]. The HL60/MX-2 line was slightly more resistant to amsacrine (65-fold) than to etoposide (51-fold; Table 2). However, HL60/MX-2 was only nine-fold cross-resistant to AMCA, indicating that topo IIβ is not the only target for AMCA.

Table 1 IC_{50} values of topoisomerase II (topo II) targeting agents on yeast JN394*t2-4* transformants. *mAMCA* methyl N-(4′-(9-acridinylamino)-2-methoxyphenyl)carbamate hydrochloride, *AMCA* methyl N-(4′-9-acridinylamino)-phenyl)carbamate hydrochloride, *DACA* N-[2-(dimethylamino)ethyl]acridine-4-carboxamide, *Cl-DACA* 7-chloro derivative of DACA, IC_{50} drug concentration reducing the number of colonies by 50% compared with cells grown in absence of drug

Acridine	Topo IIα (μ*M*)	Topo IIβ (μ*M*)	Yeast topo II (μ*M*)
mAMCA	3.5	4.0	4.0
AMCA	22	20	22
Amsacrine	15	19	73
DACA	55	>137	>137
Cl-DACA	90	>125	>125

The effect of variation in the amount of active topo IIα on cytotoxicity in mammalian cells was investigated using a panel of drug-resistant cell lines. The four mutant CHO cell lines used had either lowered levels of topo IIα or a mutated form of it. The $VPMR_5$ line was selected in teniposide [19] and expresses a fully active topo IIα that is unresponsive to drug, and that has an arginine to glutamine mutation at amino acid 493 [7]. The MTZ_4 line was selected in mitoxantrone and appears to have one unchanged topo IIα allele and the other with a cysteine to tyrosine mutation at amino acid 426 [30]. The SMR_5 cell line was selected in etoposide and has a five-fold reduction in the level of topo IIα [36]. The SMR_{16} line was also selected in etoposide and has reduced topo IIα activity with the purified enzyme being sensitive to etoposide [32].

Clonogenicity results are shown in Fig. 4, and IC_{50} values and resistance factors are shown in Table 2. The $VPMR_5$ cells were 17-fold resistant to etoposide, but only 4.9- and 2.5-fold resistant to amsacrine and AMCA, respectively. The other mutant cell lines (MTZ_4, SMR_5, SMR_{16} and H209/V6) showed a similar pattern of higher resistance to etoposide than to amsacrine or AMCA. The SMR_{16} cells were 6.7-fold resistant to amsacrine and 3.2-fold to AMCA. The human H209/V6

Table 2 Cytotoxicity data for mammalian cell lines. Data for the Chinese hamster ovary (*CHO*) cell lines were based on a 4-h drug exposure and 50% viability values from the clonogenic assays in Fig. 4. Data for the human lines were based on a 72-h drug exposure and measurement of IC_{50} values by MTT assay. *AMCA* methyl N-(4′-9-acridinylamino)-phenyl)carbamate hydrochloride, *IC_{50}* drug concentration reducing the number of colonies by 50% compared with cells grown in absence of drug

Cell line	Etoposide		Amsacrine		AMCA	
	IC_{50} (μ*M*)	Resistance factor	IC_{50} (μ*M*)	Resistance factor	IC_{50} (μ*M*)	Resistance factor
CHO WT	3.38	1.0	0.083	1.0	0.172	1.0
CHO/$VPMR_5$	57.6	17.0	0.405	4.9	0.425	2.5
CHO/MTZ_4	62.4	18.5	0.793	9.6	0.726	4.2
CHO/SMR_5	15.3	4.5	0.189	2.3	0.292	1.7
CHO/SMR_{16}	85.1	25.2	0.553	6.7	0.551	3.2
H209	0.185	1.0	0.027	1.0	0.218	1.0
H209/V6	4.77	25.8	0.236	8.7	0.620	2.8
HL60	0.093	1.0	0.011	1.0	0.118	1.0
HL60/MX-2	4.72	50.8	0.715	65.0	1.00	8.5

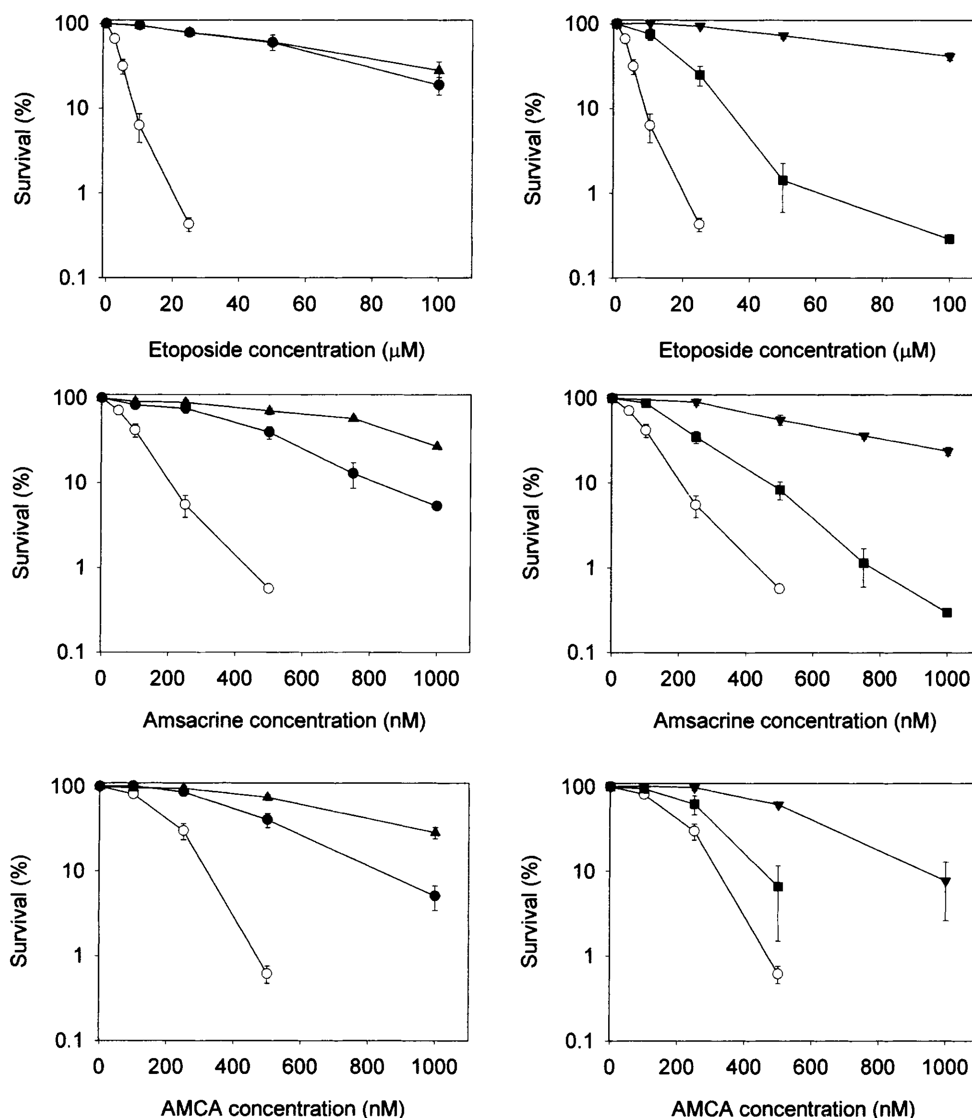

Fig. 4 Survival curves for CHO WT line (○), $VPMR_5$ cells (*left panels*, ●), MTZ_4 cells (*left panels*, ▲), SMR_5 cells (*right panels*, ■) and SMR_{16} (*right panels*, ▼). Curves for etoposide, amsacrine and AMCA are shown on the top, middle and lower graphs, respectively. Error bars indicate the standard deviation from the mean. *AMCA* methyl N-(4′-9-acridinylamino)-phenyl)carbamate hydrochloride, *CHO* Chinese hamster ovary

cell line, selected in etoposide, has an unaltered topo IIβ [13] but the topo IIα exists in a truncated form that is unable to enter the nucleus [23]. The H209/V6 cells showed a pattern similar to the SMR_{16} CHO cells, being highly resistant (26-fold) to etoposide and more resistant to amsacrine (8.7-fold) than to AMCA (2.8-fold).

Discussion

We have considered here whether the activity of the carbamate derivatives AMCA and mAMCA is mediated by interaction with topo IIβ. Studies in a cell-free system using human topo IIα and β purified from yeast indicate that both enzymes are poisoned by AMCA and mAMCA. We have also used a heterologous yeast assay to determine the drug sensitivity of human topo IIα, human topo IIβ and yeast topo II to AMCA and mAMCA, and have compared them with two other acridine derivatives, DACA and Cl-DACA. The work reported here suggests that either human topo IIα or β can act as a drug target for acridines in this yeast system. The IC_{50} values (Table 1) illustrate the two main conclusions. First, irrespective of whether human topo IIα, β, or yeast topo II was expressed in JN394t2-4, mAMCA was the most cytotoxic of the acridines tested. Indeed it was more cytotoxic than either amsacrine or doxorubicin tested in the same system [22]. Based on both the present results and previous studies [22], topo II poisons can be assigned to two classes in terms of their differential inhibition of TOP2α, TOP2β and yeast TOP2 transformants:

1. Those which display similar killing toward TOP2α and TOP2β transformants, namely AMCA, mAMCA and amsacrine
2. Those for which JN394t2-4 expressing the human topo IIα isoform is the most sensitive, namely DACA and Cl-DACA, doxorubicin, etoposide and mitoxantrone

None of the drugs we have tested preferentially inhibits yeast expressing human topo IIβ, but the drug merbarone is selective for yeast topo II [22].

We have also used a selection of mammalian drug-resistant cell lines to investigate the effects of AMCA. The role of topo IIβ was investigated with HL60/MX-2 line [20]. The resistance factors for amsacrine and AMCA are higher than in the other lines while that for etoposide is comparable (Table 2). The results suggest that deletion of the topo IIβ function affects the cytotoxicity of acridine derivatives more than that of etoposide. This result is consistent with findings using a CHO line DC-3F/9-OH-E, which is resistant to 9-hydroxyellipticine [12]. This line, like the HL60/MX-2 line, does not express topo IIβ and expresses reduced amounts of the topo IIα isoform. It is highly resistant to both etoposide and amsacrine. Transfection of the cell line with the gene for topo IIβ results in significant restoration of sensitivity to amsacrine but little to etoposide, suggesting amsacrine targets topo IIβ. Our results with HL60/MX-2 line suggest AMCA also targets topo IIβ, but that this is not its only target.

To investigate the role of topo IIα, five cell lines with decreased levels of active topo IIα, as well as exponential and plateau phase LLTC cells, were studied. Two CHO cell lines had reduced levels of topo IIα (SMR_5 and SMR_{16}), two CHO cell lines had mutated topo IIα ($VPMR_5$ and MTZ_4) and a human cell line (H209/V6) had a truncated form of topo IIα. All these lines ($VPMR_5$, MTZ_4, SMR_5, SMR_{16} and H209/V6) were more resistant to amsacrine than AMCA. Plateau-phase LLTC cells, expressing low amounts of topo IIα, were much more resistant to amsacrine than to AMCA or mAMCA. They are also highly resistant to DACA [16].

In conclusion, our results demonstrate that the carbamate analogues of amsacrine, as well as amsacrine itself, target both α and β isoforms of topo II both in yeast and in mammalian cell lines. However, a decrease in the α isoform in mammalian cells, resulting from either mutation or from growth to high density, attenuates the cytotoxicity of amsacrine to a greater effect than that of AMCA or m-AMCA. An understanding of the reasons for this difference between these two systems may not only improve our understanding of how topo II functions in cells, but might also lead to new therapeutic strategies based on the targeting of topo II in slowly growing cells.

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