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ORIGINAL ARTICLE

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Differences in the longevity of topo IIα and topo IIβ drug-stabilized cleavable complexes and the relationship to drug sensitivity

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Abstract *Purpose*: DNA topoisomerase II (topo II) is an important cellular target for chemotherapeutic agents. Human cells have two isoforms of topo II (α and β), and both are inhibited by the chemotherapeutic agents etoposide, amsacrine (mAMSA) and mitoxantrone. It is known that the cytotoxic importance of topo IIα or topo IIβ drug-induced complexes differs depending on which drug is present. This study was designed to (a) assess isoform-specific formation and reversal of topo IIα and β cleavable complexes, and (b) determine whether the cytotoxic importance of either isoform was related to differences in the longevity of the complexes. *Methods*: Mouse embryonic fibroblasts (MEFs) were used to study the cellular response to the topo II poisons etoposide, mitoxantrone and mAMSA. The longevity of topo IIα and β complexes was determined using the TARDIS assay. This immunofluorescence assay can differentiate between the topo II isoforms and thus allowed us to investigate the persistence and importance of topo IIα and β complexes for the first time. *Results*: In MEFs treated with etoposide, 50% of topo IIα complexes dissociated within 40 min whereas dissociation of topo IIβ complexes took only 20 min. Disappearance of complexes was a slower process for mitoxantrone-treated cells. The time taken to reduce topo IIα and topo IIβ cleavable complexes by 50% was 10 and 6 h, respectively. In contrast, mAMSA-stabilized topo IIα and topo IIβ cleavable complexes were equally stable (dissociation within 15 min for both isoforms). These stability data were confirmed using an in vitro assay. *Conclusions*: We previously demonstrated that topo IIα is the major target for etoposide and mitoxantrone but that both topo IIα and topo IIβ are important for mAMSA cytotoxicity. The longevity of the topo IIα and β cleavable complexes shown here is therefore an important factor in determining the cytotoxic sensitivity of either isoform to these drugs.

Keywords DNA · Topoisomerase II · Etoposide · mAMSA · Mitoxantrone · Cleavable complex

Abbreviations *FITC* Fluorescein isothiocyanate · *mAMSA* Amsacrine · *PBS* Phosphate-buffered saline · *TARDIS* Trapped in agarose DNA immunostaining · *topo* Topoisomerase

Introduction

Eukaryotic topoisomerase II (topo II) is an ATP-dependent nuclear enzyme that is essential for regulating DNA topology. It functions as a dimer and catalyses the passage of one DNA helix through another by creating a transient double-strand break. During DNA cleavage, each topo II monomer becomes covalently bound to the 5′-phosphate group of DNA via a tyrosine residue. This covalent intermediate is often termed a "cleavable complex". Topo II activity is required for a number of cellular functions including DNA replication and chromosome organization [22]. Additionally, topo II is also an important cellular target for chemotherapeutic agents [16, 25].

Two isoforms of topo II are found in mammals, topo IIα (170 kDa) and topo IIβ (180 kDa), and in humans their genes are located on separate chromosomes, 17q21–22 and 3p24, respectively [21]. Topo IIα and topo IIβ share 78% amino acid identity within the N-terminal three-quarters. The C-terminal quarter is less homologous, sharing only 34% identity [2]. Both isoforms are differentially regulated during the cell cycle and are

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thought to carry out specific cellular functions (see reference 1 and references therein).

Chemotherapeutic drugs that target topo II (topo II poisons) inhibit the normal cellular function of the enzyme by stabilizing the cleavable complex that is usually only transiently formed during the enzyme's catalytic cycle. Some of the drug-stabilized topo II-associated DNA breaks are thought to undergo cellular processing to create more lethal DNA lesions which can either be repaired or initiate cell death [6, 17, 20].

Drug-stabilized cleavable complexes can dissociate upon drug removal [14]. The topo II poisons etoposide, teniposide and mAMSA all form relatively short-lived complexes which dissociate within 2 h of drug removal [10, 15]. However, other compounds such as doxorubicin, idarubicin and mitoxantrone form complexes that are much longer lived. The longevity of drug-stabilized cleavable complexes could be related to drug potency with compounds such as mitoxantrone and doxorubicin, which form more stable complexes, and are more potent than etoposide and mAMSA, whose complexes dissociate more rapidly [3, 10, 23, 24].

Previously we have shown that etoposide, mAMSA and mitoxantrone stabilize both topo IIα and topo IIβ cleavable complexes in vivo, in mTOP2β-4 wild-type mouse embryonic fibroblasts (MEFs) [9]. Cytotoxicity studies comparing the survival of mTOP2β-4 topo $II\beta^{+/+}$ cells and mtop2β-5 topo $II\beta^{-/-}$ cells (which lack topo IIβ) have shown that both cell lines have similar sensitivities to etoposide and mitoxantrone (although at concentrations 10- to 100-fold higher than the IC_{50}, the knockout cells are slightly more resistant). In contrast, after treatment with mAMSA and other acridine derivatives the topo $II\beta^{-/-}$ cells are significantly more drug resistant over the full range of drug concentrations used. These results suggest that although drug-stabilized topo IIα and topo IIβ cleavable complexes are formed with all three compounds, topo IIα is the main cytotoxic target of etoposide and mitoxantrone, whereas both topo IIα and topo IIβ are important for the cytotoxic effects of mAMSA (and the other acridines). Cytotoxicity data also confirm that mitoxantrone is the most potent compound, followed by mAMSA and then etoposide [9], in agreement with previously published data [12, 13].

Unlike previously used techniques (e.g. alkaline elution) the TARDIS assay has enabled us to investigate isoform-specific formation and reversal of topo IIα and β cleavable complexes in wild-type MEFs after treatment with etoposide, mAMSA and mitoxantrone. These studies allowed us to determine whether the cytotoxic importance of either isoform was related to specific differences in the longevity of the complexes.

Materials and methods

Maintenance of murine cell lines and drug treatment

Wild-type mTOP2β-4 MEFs (containing both topo IIα and topo IIβ) were kindly provided by J.C. Wang, Harvard University, Cambridge, USA. They were grown as monolayers at 37°C in a humidified atmosphere containing 5% CO_2 using Dulbecco's modified Eagle's medium supplemented with 10% v/v fetal bovine serum and penicillin (50 μg/ml)/streptomycin (50μg/ml) (Gibco-BRL). Cells were tested regularly for mycoplasma infection and found to be free from contamination. For drug treatment, cells were seeded at 3×10^4 cells/well into six-well tissue culture plates and drug was added to exponentially growing cells at appropriate concentrations (solvent controls were used).

Drugs were obtained from Sigma (Poole, UK) and were dissolved in methanol (etoposide), water (mitoxantrone) or dimethyl sulphoxide (mAMSA).

TARDIS (trapped in agarose DNA immunostaining)

Slide preparation

This has been described in detail by Willmore et al. [23]. In brief, control (untreated) and drug-treated cells were suspended in low melting point agarose and spread onto microscope slides that had been precoated with agarose. After solidification of the agarose by placing the slides on a cold surface the cells were lysed by placing the slides in lysis buffer containing protease inhibitors (30 min). At this stage the slides could be stored at −20°C in PBS containing 10% v/v glycerol. Proteins that were not covalently bound to the DNA were then removed by placing the slides in 1 *M* NaCl plus protease inhibitors (30 min). Topo II that was covalently bound to the DNA of each cell was then detected using two isoform-specific anti-topo II polyclonal antisera. Antiserum 18511(α) was raised in rabbits to full-length recombinant human topo IIα and antiserum 18513(β) was raised in rabbits to the recombinant C-terminal domain of human topo IIβ; these antibodies were specific for topo IIα and β, respectively [8]. Antiserum 18511(α) was used at a dilution of 1:50 and 18513(β) was used at a dilution of 1:200. Both antisera were diluted in PBS containing 0.1% v/v Tween 20 and 1% w/v bovine serum albumin. After primary staining the slides were exposed to a FITC-labelled secondary antibody (anti-rabbit FITC-conjugated secondary antibody, $F(ab')_2$ fragment; Sigma) to allow quantification of topo IIα and β cleavable complexes.

Quantification of cleavable complex levels

This has been described in detail by Frank et al. [11]. In brief, slides were stained with Hoechst 33258 (10 μ*M* in PBS; Sigma) for 5 min, and coverslips were applied and secured. Images of blue (Hoechst-stained DNA) fluorescence and green (FITC-stained topo IIα and β) immunofluorescence were captured using an epifluorescence microscope and a cooled slow-scan charged-coupled device (CCD) camera.

Images were analysed to quantify the levels of Hoechst fluorescence and FITC immunofluorescence using Visilog 4 software (Neosis). All images were corrected for stray light and camera background. Additionally, images were subjected to blue and green shade correction to compensate for variation in intensity of illumination and nonuniformities in light transmission [11, 23]. Statistical analysis was carried out using GraphPad Prism software (www.graphpad.com).

Calculating the dissociation of topo IIα and β cleavable complexes

Mean FITC immunofluorescence values from each independent experiment were used to calculate the percentage dissociation of topo IIα and topo IIβ cleavable complexes. The maximal FITC immunofluorescence value from cells treated with the highest drug dose and collected immediately after drug treatment was taken as 100%. Values from cells collected at different time-points or treated with lower drug doses were expressed as a percentage of maximal immunofluorescence.

Reversal of drug-induced cleavable complexes in vitro

A 500-bp fragment of pBR322 was cleaved by purified topo IIα or topo IIβ in the presence of 50 μ*M* etoposide or mAMSA, and reversal of cleavable complexes using salt was assessed using the method described in reference 19.

Results

Dissociation of etoposide-stabilized topo IIα and topo IIβ cleavable complexes

The TARDIS assay was used to examine the dissociation rate of etoposide-stabilized topo IIα and topo IIβ cleavable complexes in wild-type MEFs. Cells were treated with 0, 10 or 100 μ*M* etoposide for 2 h. After drug treatment cells were either collected immediately and placed on ice or placed in prewarmed drug-free medium for 30, 60 or 120 min at 37°C. Control and drug-treated cells were embedded in agarose and slides were stained with either 18511(α) for detection of topo IIα complexes, or 18513(β) for detection of topo IIβ complexes.

Typical images showing levels of Hoechst and FITC immunofluorescence for cells treated with 100 μ*M* etoposide are shown in Fig. 1. Levels of blue Hoechst-stained DNA fluorescence did not change following drug treatment and removal (Fig. 1a–d, i–l). In contrast, levels of FITC immunofluorescence associated with each cell varied depending on the treatment schedule. In the absence of etoposide, immunofluorescence was undetectable after staining with both 18511(α) and 18513(β) (data not shown), as previously reported [9, 23].

Levels of immunofluorescence were maximal in cells treated with 100 μ*M* etoposide when collected immediately after drug exposure, shown in Fig. 1e for topo IIα and Fig. 1m for topo IIβ. Figure 1f–h shows FITC immunofluorescence levels associated with topo IIα cleavable complexes after 30, 60 or 120 min in drug-free medium, respectively. These images demonstrate a time-dependent reduction in the levels of FITC immunofluorescence. Similarly, levels of immunofluorescence associated with topo IIβ cleavable complexes decrease with time (Fig. 1n–p). Furthermore, topo IIβ complexes appeared to dissociate more rapidly than those formed with topo IIα as detectable levels of immunofluorescence were still observed in cells stained with 18511(α) after 1 h in drug-free medium, but not after staining with 18513(β). The reduced intensity of the FITC immunofluorescence corresponded with a decrease in both topo IIα and topo IIβ etoposide-stabilized cleavable complexes. As little or no immunofluorescence was found associated with individual cells after 2 h in drug-free medium, it is likely that most of the drug-stabilized cleavable complexes had dissociated during that time.

Levels of Hoechst fluorescence and FITC immunofluorescence from mTOP2β-4 cells treated with 100 μ*M* etoposide and placed in drug-free medium for various lengths of time were quantified for several fields of view

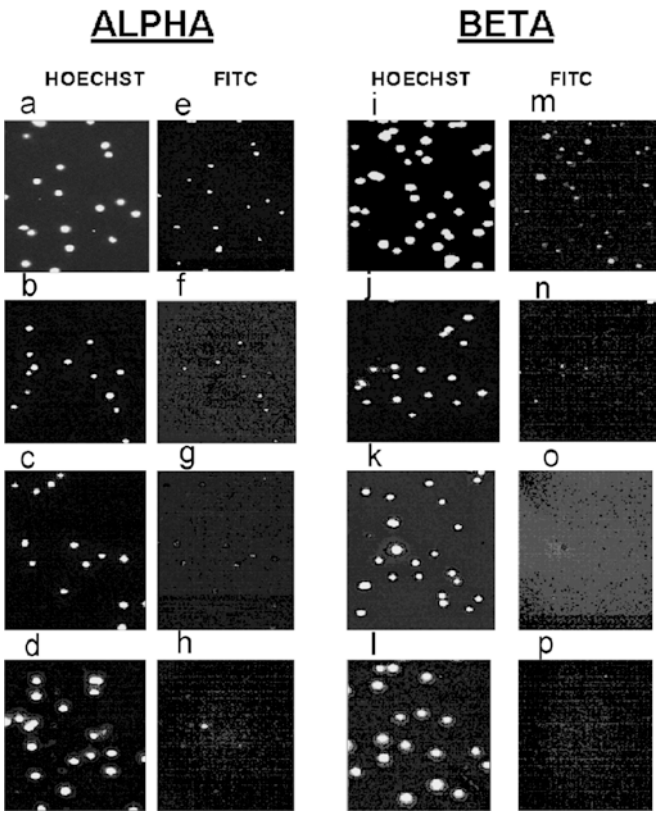

Fig. 1a–p Typical images illustrating levels of Hoechst and FITC immunofluorescence after mTOP2β-4 cells were treated with 100 μ*M* etoposide. **a–d**, **i–l** Hoechst fluorescence from mTOP2β-4 cells treated with 100 μ*M* etoposide and placed in drug-free medium for 0, 30, 60 or 120 min, respectively; **e–h**, **m–p** associated FITC immunofluorescence from individual cells. Staining with 18511(α) and 18511(β) was used to detect topo IIα (**a–h**) and topo IIβ (**i–p**) cleavable complexes, respectively

(> 80 cells) and data from a typical experiment are shown in Fig. 2. Figure 2a–d confirms that there were no changes in Hoechst fluorescence after drug removal. However, Fig. 2e–h clearly demonstrates the decrease in FITC immunofluorescence associated with both topo IIα and topo IIβ cleavable complexes with increasing lengths of time in drug-free medium.

FITC immunofluorescence data for both topo IIα and topo IIβ (from at least three independent experiments) were combined by expressing the mean integrated fluorescence intensity at each time-point as a percentage of the maximal FITC immunofluorescence value obtained from cells treated with 100 μ*M* and collected immediately after drug treatment (Fig. 3). This analysis confirmed that topo IIβ cleavable complexes dissociated more rapidly than those formed with topo IIα. Furthermore, there was a significant difference in the longevity of the topo IIα and topo IIβ cleavable complexes, with the time taken for 50% of the topo II cleavable complexes to dissociate being approximately 40 min for topo IIα and 20 min for topo IIβ ($P = 0.0019$, ANOVA). Overall, etoposide-stabilized topo IIα cleavable complexes were approximately twofold more stable

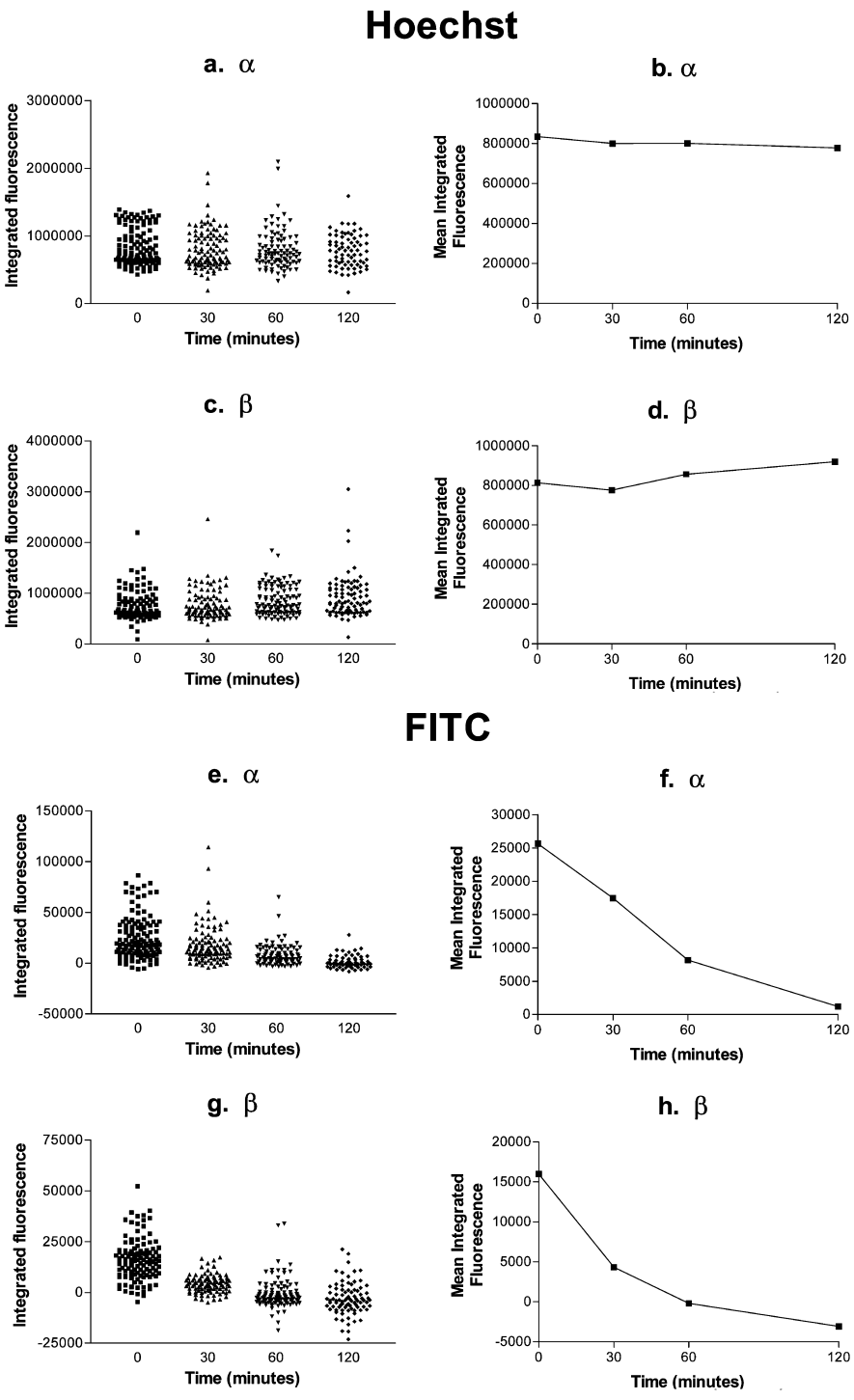

Fig. 2a–h Hoechst fluorescence and FITC immunofluorescence values associated with individual cells and the corresponding mean integrated fluorescence values. The data presented are from one typical experiment in which mTOP2β-4 cells were treated with 100 μM etoposide for 2 h and placed in drug-free medium for 0, 30, 60 or 120 min. **a, c, e, g** Scattergrams showing levels of Hoechst or FITC fluorescence after staining with either 18511(α) or 18513(β), as indicated. **b, d, f, h** Plots of the corresponding mean integrated Hoechst or FITC fluorescence values

than those formed with topo IIβ and most topo II etoposide-stabilized complexes had dissociated within 2 h of drug removal.

Dissociation of mitoxantrone-stabilized topo IIα and topo IIβ cleavable complexes

Similarly, the TARDIS assay was used to determine the stability of topo IIα and topo IIβ cleavable complexes after treatment with 0, 0.1 or 1 μM mitoxantrone, for 2 h. Cells were collected 0, 1, 6, 16 or 24 h after removal of the mitoxantrone and levels of FITC immunofluorescence associated with topo IIα and topo IIβ cleavable complexes were analysed by staining with 18511(α) or 18513(β).

Figure 4a, b shows the dissociation of mitoxantrone-stabilized topo IIα and topo IIβ cleavable complexes, respectively. After 1 h in drug-free medium there was no detectable decrease in topo IIα complexes. However, after

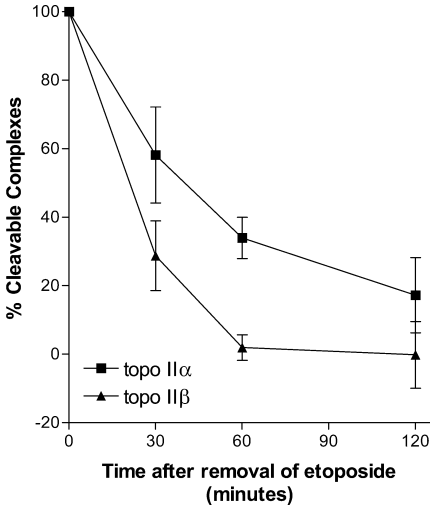

Fig. 3 Comparison of the dissociation of etoposide-stabilized topo IIα and topo IIβ cleavable complexes with time. mTOP2β-4 cells were treated with 100 μ*M* etoposide for 2 h and collected 0, 30, 60 and 120 min after growth in drug-free medium. The data are presented as percentages of the total FITC immunofluorescence from cells collected immediately after drug treatment, and each value is the mean ± SE of at least three independent experiments

more time in drug-free medium a reduction in topo IIα cleavable complexes was observed. In contrast, topo IIβ complexes had decreased slightly within 1 h after drug removal and further reductions were observed with longer periods of time in drug-free medium. After 24 h the levels of FITC immunofluorescence were similar to background levels (observed in untreated control cells) for both topo IIα and topo IIβ suggesting that most of the topo II-DNA complexes had dissociated within 24 h.

To examine the dissociation of topo IIα and topo IIβ cleavable complexes, the dissociation rates were compared by expressing them as a percentage of the maximal levels of FITC immunofluorescence obtained when cells were collected immediately after treatment with 1 μ*M* mitoxantrone (Fig. 4c). The time taken to reduce topo IIα and topo IIβ cleavable complexes by 50% was 10 and 6 h, respectively. Therefore topo IIα cleavable complexes were 1.7-fold more stable than topo IIβ complexes ($P = 0.1392$, ANOVA).

Dissociation of mAMSA-stabilized topo IIα and topo IIβ cleavable complexes

mTOP2β-4 cells were treated with 0, 1 or 2.5 μ*M* mAMSA for 2 h. Cells were then either collected immediately or placed in drug-free medium for 0, 15, 30, 60 or 120 min. FITC immunofluorescence data from mTOP2β-4 cells stained with 18511(α) and 18513(β) are shown in Fig. 5a, b, respectively. For both topo IIα and topo IIβ there was a time-dependent reduction in FITC immunofluorescence after treatment with mAMSA, demonstrating dissociation of both topo IIα and topo IIβ complexes with time. Furthermore, after 2 h in drug-free medium, levels of fluorescence associated with topo IIα and topo IIβ complexes were similar to those observed in untreated control cells suggesting that most complexes had dissociated within 2 h.

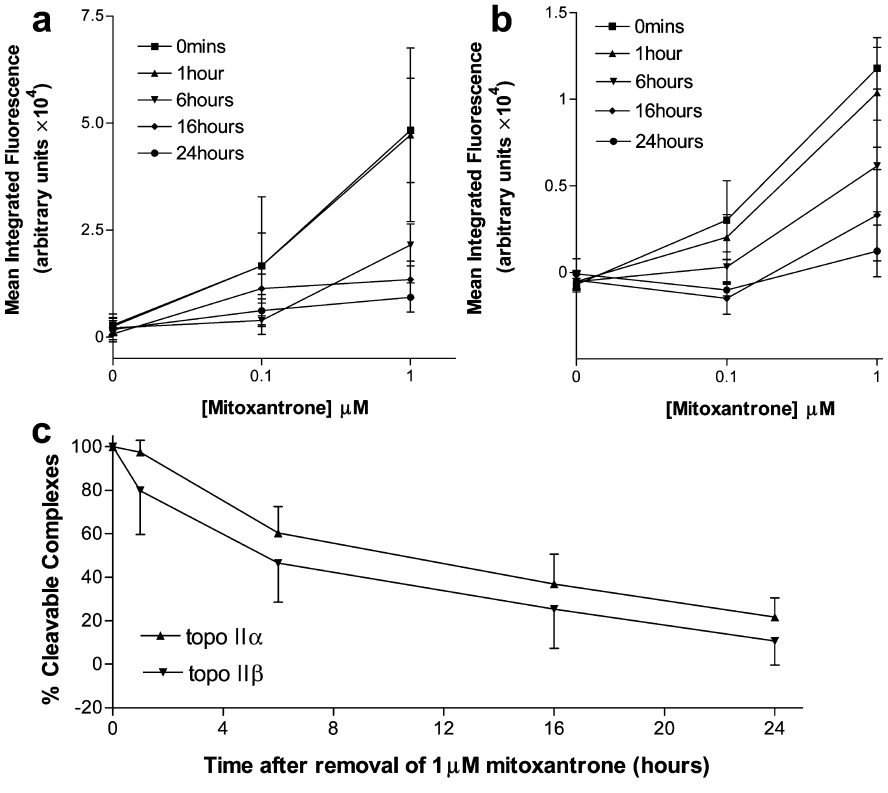

Fig. 4a–c Dissociation of mitoxantrone-stabilized topo IIα and topo IIβ cleavable complexes with time. mTOP2β-4 cells were treated with 0, 0.1 or 1 μ*M* mitoxantrone for 2 h. Cells were collected immediately after drug exposure or following 1, 6, 16 or 24 h in drug-free medium. Mean integrated immunofluorescence levels from cells stained with either (**a**) 18511(α) or (**b**) 18513(β). **c** Dissociation of topo IIα and topo IIβ cleavable complexes after treatment with 1 μ*M* mitoxantrone. These data are percentages of the total FITC immunofluorescence produced in cells treated with 1 μ*M* mitoxantrone that had been collected immediately after drug treatment, and each value is the mean of at least three independent experiments with the SE shown in one direction for clarity

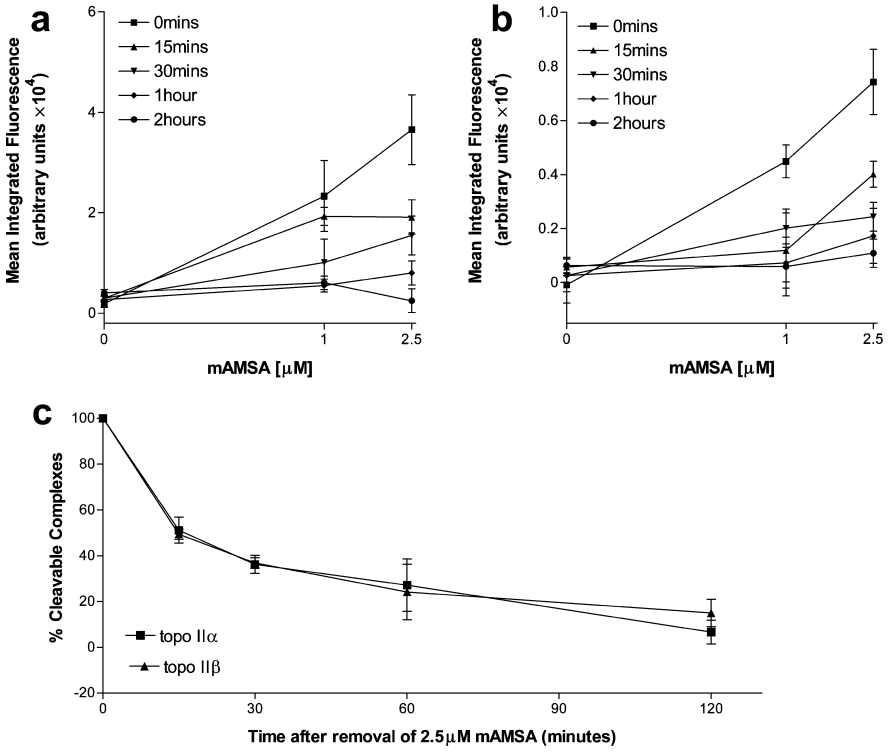

Fig. 5a–c Dissociation of mAMSA-stabilized topo IIα and topo IIβ cleavable complexes with time. mTOP2β-4 cells were treated with 0, 1 or 2.5 μ*M* mAMSA for 2 h. Cells were collected immediately after drug exposure or after 15, 30, 60 or 120 min in drug-free medium. Mean integrated immunofluorescence levels from cells stained with either (**a**) 18511(α) or (**b**) 18513(β) are shown. **c** Dissociation of topo IIα and topo IIβ cleavable complexes after treatment with 2.5 μ*M* mAMSA. These data are percentages of the total FITC immunofluorescence produced in cells treated with 2.5 μ*M* mAMSA that had been collected immediately after drug treatment, and each value is the mean ± SE of at least three independent experiments

FITC immunofluorescence values were converted to a percentage of maximal levels obtained from cells treated with 2.5 μ*M* mAMSA and collected immediately after drug exposure (Fig. 5c). The time taken for 50% of the complexes to dissociate was approximately 15 min for both isoforms.

Reversal of drug-induced cleavable complexes in vitro

In order to study the kinetics of complex reversal for purified topo II enzymes, a 500-bp fragment of pBR322 was cleaved by purified topo IIα or topo IIβ in the presence of etoposide or mAMSA. The reversibility of these complexes was assessed to determine the in vitro stability of the complexes with each isoform. Results for the salt reversal of mAMSA-induced complexes showed that both topo IIα and topo IIβ complexes had fully reversed by 30 min (Fig. 6). Kinetics for etoposide-induced complexes showed distinct differences between the isoforms. Approximately half of the topo IIβ complexes had reversed by 20 min, whereas the topo IIα complexes remained even after 30 min. These data confirm that the kinetics of reversal for mAMSA and etoposide seen using the TARDIS assay are analogous to those obtained using this in vitro assay system.

Discussion

We studied the dissociation of topo IIα and topo IIβ cleavable complexes following drug removal using the TARDIS assay. We propose that differences in the stability of topo II α and topo IIβ cleavable complexes are related to the cytotoxic importance of each isoform and may be a determinant for drug potency. Our data also confirm previous reports that the longevity of topo II drug-stabilized cleavable complexes varies depending on the drug used.

Stability of topo II α and topo IIβ complexes following drug removal

In this study both topo IIα and topo IIβ complexes formed by etoposide in MEFs dissociated within 2 h of drug removal, in agreement with our earlier data in human leukemic CCRF-CEM cells [23]. We also observed loss of mAMSA-induced topo IIα and topo IIβ complexes within 2 h of drug removal.

These data for topo IIα and topo IIβ are in agreement with earlier studies on topo II that did not differentiate between the two isoforms, in that they showed reversal of complexes within 2 h of drug removal for both etoposide and mAMSA. For example, alkaline elution studies in human lung adenocarcinoma (A549) cells have demonstrated that DNA single-strand breaks (SSBs) and double-strand breaks (DSBs) induced by etoposide are almost completely removed within 2 h [15]. Zwelling et al. [24] and Covey et al. [7] have also shown that mAMSA-induced SSBs, DSBs and DNA-protein crosslinks almost completely disappear within 60 min of drug removal. Furthermore, Caldecott et al. [4] have found that etoposide and mAMSA-induced protein-DNA crosslinks and DSBs all dissociate within 2 h of drug removal in CHO-K1 cells.

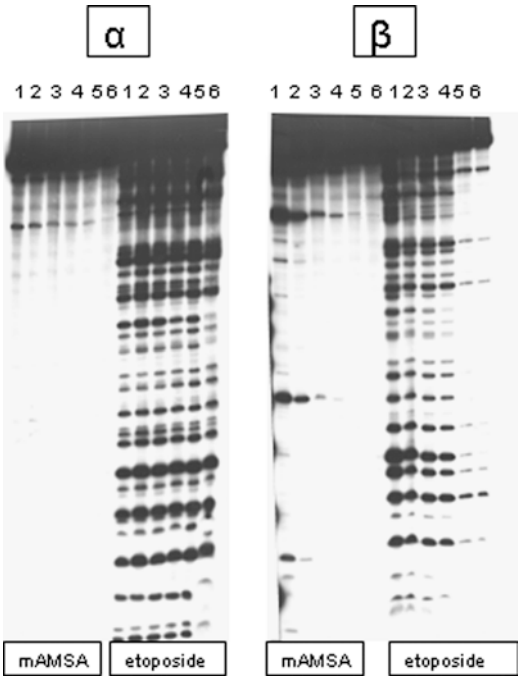

Fig. 6 Reversal of drug-induced cleavable complexes in vitro. A pBR322 fragment was cleaved by purified topo IIα or topo IIβ in the presence of 500 μ*M* etoposide or mAMSA, and cleavage after 0 (*lane 1*), 2 (*lane 2*), 5 (*lane 3*), 10 (*lane 4*), 20 (*lane 5*) or 30 min (*lane 6*) of reversal time was assessed. *Right panel* shows the cleavage pattern for topo IIα and the *left panel* the cleavage pattern for topo IIβ after treatment with mAMSA or etoposide

In contrast to etoposide and mAMSA, mitoxantrone-induced complexes appeared to be much more stable, still being detected up to 24 h after drug removal for both topo IIα and topo IIβ complexes (Fig. 4). Previous non isoform-specific investigations in KG1a, Tf1, HL-60 and U937 cell lines have shown that mitoxantrone-induced topo II complexes are more persistent than those of etoposide and mAMSA. This prolonged stability of the topo II complexes in the four cell lines may be due to mitoxantrone retention in the cells after drug removal (see, for example, reference 3) and could be due to an equilibrium between old and new complexes, with new complexes being stabilized by mitoxantrone retained within the cell.

Half-lives of topo IIα and topo IIβ complexes

Following etoposide exposure, topo IIα complexes were two-fold more stable than topo IIβ complexes, with half-lives of 40 and 20 min, respectively. Similarly, the topo IIα complexes formed by mitoxantrone treatment were 1.7-fold more stable than the topo IIβ complexes, with half-lives of 10 and 6 h, respectively. In contrast to etoposide and mitoxantrone, mAMSA-stabilized topo IIα and topo IIβ cleavable complexes had identical dissociation rates after drug removal, with half-lives of 15 min for both isoforms. Similar results were obtained when an in vitro cleavage assay (Fig. 6) was used to determine reversal of etoposide- or mAMSA-induced complexes. These results confirmed the different kinetics of reversal for etoposide and mAMSA seen using the TARDIS assay and suggest that each drug may have distinct affinities for topo IIα and for topo IIβ.

Relationship between cytotoxicity and the stability of α and β complexes

Although etoposide and mitoxantrone induced both topo IIα and topo IIβ cleavable complexes, topo IIα appeared to be the major cytotoxic target of these compounds. Previous data show that $\beta^{-/-}$ MEFs are resistant (compared to $\beta^{+/+}$ MEFs) but only at the highest doses of etoposide or mitoxantrone [9]. The longer half-life of the topo IIα complexes could explain the greater importance of topo IIα for etoposide- and mitoxantrone-induced cytotoxicity. The increased persistence of topo IIα complexes would allow further cellular processing of the usually reversible topo II-associated break into a more permanent DNA lesion and subsequently enhance the sensitivity of this isoform.

In contrast, $\beta^{-/-}$ MEFs showed resistance at all of the concentrations of mAMSA analysed when compared to $\beta^{+/+}$ MEFs, demonstrating the importance of both topo IIα and topo IIβ for the cytotoxic effects of mAMSA [9]. Since topo IIα and topo IIβ mAMSA-induced complexes were equally stable, this could explain why both isoforms were important for the cytotoxic effects of this compound.

The study on the $\beta^{-/-}$ MEFs also showed mitoxantrone to be more potent than mAMSA and etoposide (IC_{50} values of 5, 60 and 1700 n*M*, respectively, [9]). The greater stability of mitoxantrone-induced complexes compared to those produced by etoposide and mAMSA may contribute to its higher potency. However, it is likely that other factors are also involved in determining drug potency. For example, the ratio of SSBs and DSBs produced and also the location of DNA damage within the genome are thought to be important [18]. Topo II cleaves DNA at specific sequences and nucleotide preferences have been identified for each topo II poison. Site-specific interactions of topo II (α and β) with the DNA in the presence of different topo II poisons could induce DNA damage at different places within the genome. This could, in turn, affect the cytotoxicity of each drug and the cytotoxic importance of topo IIα and topo IIβ (reviewed in reference 5).

In conclusion, our results indicate that differences in the stability of topo IIα and topo IIβ cleavable complexes plays a role in determining the importance of each isoform in cytotoxicity. In addition, we confirmed that the longevity of topo II stabilized-cleavable complexes is drug-dependent, with complexes formed by mitoxantrone being the most persistent. The longevity of drug-induced cleavable complexes may have implications for administration of these agents in the clinic, since one would predict that the use of a topo II agent that

induced long-lived complexes would allow dose reduction, possibly reducing patient toxicity.

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