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Enhancing the antiproliferative effect of topoisomerase II inhibitors using a polypeptide inhibitor of c-Myc

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Abbreviations:
aa, amino acid
BSA, bovine serum albumin
BrdU, 5-bromo-2′-deoxyuridine
DFMO, α-1,2 difluoromethylornithine
ELP, elastin-like polypeptide
H1, helix 1 of the c-Myc helix-loop-helix domain
ODC, ornithine decarboxylase
Pen, penetratin peptide
PBS, phosphate-buffered physiological saline
RT-PCR, reverse transcriptase polymerase chain reaction
T_t, transition temperature

ABSTRACT

Topoisomerase II inhibitors are widely used in cancer chemotherapy. However, their use is limited by severe adverse effects to normal tissues, including cardiotoxicity. One approach to reduce the cytotoxicity in normal tissues may be to sensitize cancer cells to the toxicity of these agents, allowing them to be administered in a lower and safer dose. A hallmark of many types of cancer is overexpression of c-Myc, and a molecule which targets c-Myc will affect the cancer cells more significantly than the normal tissues. This report demonstrates that pretreatment of cells with a polypeptide, which inhibits c-Myc transcriptional function causes cells to be more susceptible to the topoisomerase II inhibitors doxorubicin and etoposide. Inhibition of c-Myc and Max dimerization by this polypeptide leads to as much as a 2-fold reduction in the doxorubicin and etoposide IC_{50} in three different cell lines tested. Furthermore, the c-Myc inhibitor affects the cell cycle distribution of MCF-7 breast cancer cells by enhancing the G_0/G_1 accumulation induced by doxorubicin and etoposide. We have shown that this effect is not due to enhanced drug accumulation or inhibited drug efflux. Rather, it is likely due to the transcriptional consequences of c-Myc inhibition, specifically reduction in the levels of the polyamine synthesizing enzyme ornithine decarboxylase. In summary, our results suggest that polypeptides, which inhibit c-Myc transcriptional function, may prove to be a useful tool in combination therapy with topoisomerase II inhibiting drugs.

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1. Introduction

Doxorubicin (DOX) is a widely used topoisomerase II inhibitor for cancer chemotherapy. However, the use of DOX is limited by severe side effects, including cardiotoxicity, and the development of resistance [1]. To overcome these limitations and improve the specificity and efficacy of DOX therapy, efforts have focused on improving DOX delivery to the tumor site by encapsulating it in liposomes [2] or by conjugating it to polymeric carriers [3]. An alternative approach is to specifically sensitize the tumor cells to systemically circulating DOX. We report here that a polypeptide inhibitor of c-Myc transcriptional function enhances the in vitro toxicity of the topoisomerase II inhibitors DOX and etoposide in the MCF-7, HeLa and MES-SA cell lines.

c-Myc is a transcription factor that provides control over cell growth, proliferation, apoptosis and tumorigenesis [4]. Expression of c-Myc is often deregulated in cancer, and thus it provides an attractive target for anticancer therapy [5]. In order to regulate transcription, c-Myc forms a heterodimer with Max [6]. Interruption of this dimerization is a potent method for interfering with c-Myc function [7,8]. Inhibition of c-Myc and Max dimerization will affect processes, such as cell growth and proliferation that are directly controlled by c-Myc [4], and they may also affect the cell's response to other drugs through c-Myc's influence on DNA repair and apoptosis [4,9].

One approach to prevent transcriptional activation by c-Myc is to block the interaction between c-Myc and Max using a c-Myc inhibitory peptide. This peptide, first described by Draeger and Mullen [10] and applied in cell culture by Giorello et al. [8], is derived from helix 1 of the helix-loop-helix region of c-Myc. In a recent report, we described the fusion of the c-Myc inhibitory peptide (H1) to a thermally responsive biopolymer called elastin-like polypeptide (ELP) [7]. ELP is a biopolymer derived from a structural motif of the mammalian elastin protein that is composed of tandem repeats of the five amino acids VPGXG, where X is any amino acid except proline [11]. Polymer carriers are advantageous for delivery of therapeutic peptides because they passively accumulate in tumors due to the enhanced permeability and retention effect [12–15], and they may lengthen the half-life of the peptide in circulation [16]. Furthermore, ELP is a thermally responsive polymer, and, in combination with hyperthermia, ELP may be targeted to the tumor site [12]. Penetratin (Pen), a peptide known to mediate translocation across cell membranes [17–19], was also added to the polypeptide to allow its efficient delivery into the cell cytoplasm. The resulting polypeptide (Pen–ELP–H1) was shown to block the interaction between c-Myc and Max, reduce the expression of genes controlled by c-Myc/Max, and inhibit proliferation in MCF-7 breast cancer cells [7].

In this study, treatment of cells with Pen–ELP–H1 is shown to enhance the antiproliferative effect of doxorubicin and etoposide. Inhibition of transcriptional activation by c-Myc enhances the DOX and etoposide induced accumulation in G_0/G_1 of the cell cycle, but has no effect on intracellular drug levels. Sensitization to topoisomerase II inhibitors is likely due to lowering the levels of the polyamine synthesizing enzyme ornithine decarboxylase (ODC), which is a c-Myc controlled gene that is down-regulated after Pen–ELP–H1 treatment.

2. Materials and methods

2.1. Polypeptide purification

Pen–ELP–H1 was purified as described previously [7]. Briefly, Pen–ELP–H1 was hyperexpressed [20] from pET25b+ in *E. coli* BLR(DE3) (Novagen, Madison, WI). Cells were lysed by sonication, and the cell debris and PEI precipitated nucleic acids were removed in subsequent centrifugations. The polypeptide phase transition was induced in the soluble cell lysate by heating the cell lysate to 44 °C and increasing the NaCl concentration to 2 M. Polypeptides were then collected by centrifugation and resuspended in phosphate buffered saline (PBS; Gibco, Carlsbad, CA). This process was repeated 3–5 times and purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

2.2. Cell culture

MCF-7 breast carcinoma cells (c-Myc and p53 positive [21,22]), HeLa cervical carcinoma cells (c-Myc positive [21] and p53 inactive [23]) and MES-SA uterine sarcoma cells (c-Myc and p53 status unknown) were used for this study. All cells lines were obtained from ATCC (Manassas, VA) and grown as a monolayer in 75 cm^2 tissue culture flasks in ATCC recommended media and passaged every 3–5 days. Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2. For experiments, cells were removed from tissue culture flasks by brief treatment with 0.05% (v/v) trypsin-EDTA (Invitrogen, Carlsbad, CA), plated in 6-well plates for flow cytometry (250,000 cells/well) or in 96-well plates for proliferation (5000 cells/well, four replicates per sample), and allowed to grow for 24 h before treatment.

2.3. IC_{50} determination

Cells were treated 24 h after plating (day 1) with 20 μM Pen–ELP–H1 or Pen–ELP for 1 h at 37 °C. The polypeptide was washed away, the cells were rinsed with media, and fresh media was replaced for 72 h. On day 4, cells were exposed to a concentration range of doxorubicin, etoposide, cisplatin or camptothecin (Sigma, St. Louis, MO). All drug exposures were for 72 h. Cell number was assessed on day 7 using the MTS cell viability assay (Promega, Madison, WI). IC_{50} curves were fit to an exponential equation using Microsoft Excel. The reported IC_{50} represents an average of at least five independent experiments, and significance was determined using an unpaired Student's *t*-test.

For DFMO proliferation experiments, cells were treated as described above with 20 μM Pen–ELP–H1 and/or 50 μM α-difluoromethylornithine (DFMO, Sigma) for 72 h. DOX (750 nM) or etoposide (200 μM) was added on day 4 for 72 h, and the cell number was assessed using the MTS assay.

2.4. Cell cycle analysis

Cells were treated as described above with 20 μM Pen–ELP–H1 on day 1 and DOX (100 nM) or etoposide (1 μM) on day 4. On day 5, cells were exposed to 10 μM 5-bromo-2′-deoxyuridine (BrdU, Sigma) for 1 h to mark cells in S-phase. Cells were rinsed and

harvested by trypsinization and fixed for 30 min on ice in 75% ethanol. Cells were suspended in 1 ml of 2 N HCl/0.5% Triton X-100 for 30 min at room temperature to denature the DNA. Cells were spun and resuspended in 1 ml of 0.1 M $Na_2B_4O_7$, pH 8.5, to neutralize the sample, then rinsed one time with PBS. Cells were suspended in 75 μl PBS + 0.5% Tween 20/1% BSA, RNase A (Sigma) was added to a final concentration of 750 μg/ml, and mouse-anti-BrdU-Alexa 488 (Molecular Probes, Eugene, OR) was added to a final concentration of 100 μg/ml. The sample was incubated overnight at 4 °C with gentle agitation. Cells were rinsed once with PBS and stained with 75 μg/ml propidium iodide (Sigma) for 30 min at room temperature. Alexa 488 fluorescence was measured in channel FL1 and propidium fluorescence was measured in channel FL3 using a Cytomics FC 500 flow cytometer (Beckman Coulter, Fullerton, CA). A scatter plot of forward scatter versus FL3 intensity was used to exclude cell debris and cell aggregates from the analysis.

2.5. *Doxorubicin uptake and efflux*

Cells were treated on day 1 with 20 μM Pen–ELP–H1 as described above. For uptake (accumulation) experiments, cells were exposed to 1 μM DOX on day 4 for 0.5–72 h, and then harvested for flow cytometry. For efflux, cells were loaded with DOX (1 μM) for 5 h, and then placed in drug-free media. Cells were harvested after various times ranging from 0.5 to 24 h for flow cytometry. Forward versus side scatter gating was used to remove cell debris from the analysis, and DOX fluorescence was measured using FL3. Each DOX histogram was a unimodal peak (n = 5000 cells), and the peak mean was normalized to propidium standard beads.

3. Results

3.1. *Effects of Pen–ELP–H1 on drug toxicity*

The ability of Pen–ELP–H1 to enhance the effectiveness of chemotherapeutic agents was tested in MCF-7 breast cancer cells. Cells were treated on day 1 with 20 μM Pen–ELP–H1 or a control polypeptide, which lacks the c-Myc inhibitory sequence (Pen–ELP) for 1 h, rinsed, and placed in fresh media for 3 days. On day 4, the cells were exposed to the drugs at various concentrations for 72 h, and viability was assessed using the MTS cell viability assay. As shown in Fig. 1A, the cytotoxicity of doxorubicin, a topoisomerase II inhibitor, is enhanced after pretreatment of the cells with the c-Myc inhibitory polypeptide ($P < 0.001$). Similar effects were seen with a different topoisomerase II inhibitor, etoposide ($P < 0.001$) (Fig. 1B). Data from the MTS assay was averaged and fit using an exponential equation in order to calculate the IC_{50} with and without pretreatment. Results of the IC_{50} calculations are shown in Table 1. The reduction in IC_{50} with

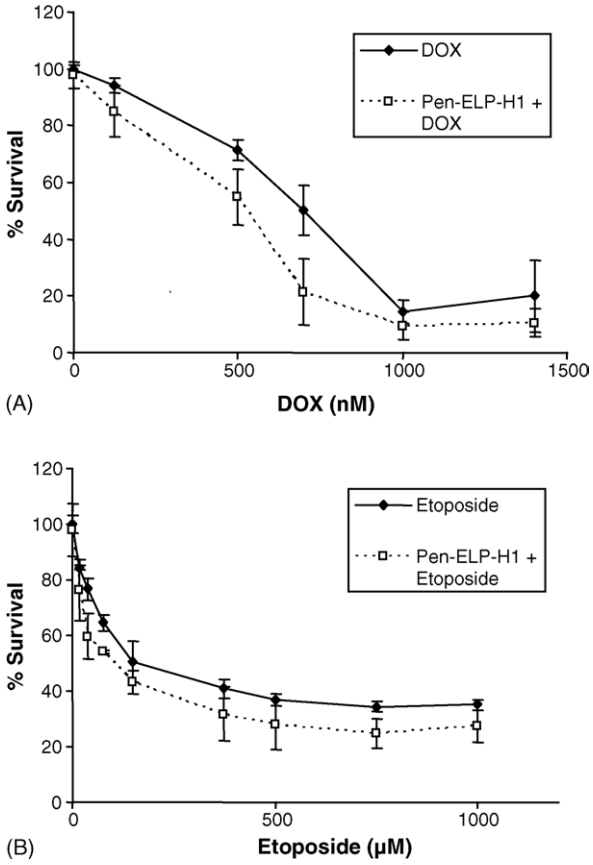


Fig. 1 – Effect of drug concentration on cell viability for DOX (A) or etoposide (B) with and without Pen–ELP–H1 pretreatment. Cells were pretreated on day 1 with 20 μM Pen–ELP–H1 and treated on day 4 with drug. Viability was determined on day 7 using the MTS cell viability assay. A single representative experiment is shown (error bars = S.D.).

Table 1 – Effects of Pen–ELP–H1 pretreatment on IC_{50} values in MCF-7 cells

Drug	IC_{50} (mean ± S.E.M.)	IC_{50} after Pen–ELP pretreatment (mean ± S.E.M.)	IC_{50} after Pen–ELP–H1 pretreatment (mean ± S.E.M.)
Doxorubicin[a] (nM)	751.7 ± 21.6	777 ± 75.1	504.2 ± 35.3
Etoposide[a] (μM)	210.4 ± 12.4	186.8 ± 13.8	141.4 ± 3.5
Camptothecin (nM)	235.1 ± 9.8	229 ± 46.4	235.4 ± 5.8
Cisplatin (μM)	10.2 ± 0.5	11.6 ± 1.6	9.4 ± 1.4

IC_{50} values were calculated from the experiment shown in Fig. 1. Curves were fit using an exponential equation, and the IC_{50} value shown represents the mean ± S.E.M. of at least five independent determinations each performed with four replicates per drug concentration. Statistical significance was assessed using an unpaired Student's t-test.

[a] Difference between control and Pen–ELP–H1 pretreated IC_{50} is statistically significant as determined by an unpaired Student's t-test ($P < 0.05$).

Pen–ELP–H1 pretreatment is about 1.5-fold for both doxorubicin and etoposide. The control polypeptide Pen–ELP had no significant effect on the DOX or etoposide IC_{50}. Also, no enhancement of the DOX and etoposide IC_{50} was observed if the treatment schedule was reversed, treating with DOX or etoposide first for 72 h, then adding Pen–ELP–H1 (data not shown). In order to examine whether the effect of c-Myc inhibition was specific to topoisomerase II inhibitors, we also tested the effect with camptothecin, a drug that inhibits topoisomerase I. Pretreatment with Pen–ELP–H1 or Pen–ELP had no significant effect on cell cytotoxicity of camptothecin. Similarly, no effect was seen on the cytotoxicity of an unrelated DNA alkylating agent, cisplatin (Table 1).

3.2. *Effects of Pen–ELP–H1 on cell cycle distribution*

To gain more insight into the effect of polypeptide and drug treatment, the cell cycle distribution of MCF-7 cells was examined. Cells were treated as above with 20 μM Pen–ELP–H1 on day 1 and doxorubicin (100 nM) or etoposide (1 μM) on day 4. On day 5, the cells were pulsed with BrdU and stained with propidium iodide for flow cytometric analysis of the cell cycle distribution. BrdU was used to accurately estimate the fraction of cells in S-phase, and this was combined with propidium iodide staining to denote each phase of the cell cycle (Fig. 2A). Treatment with the c-Myc inhibitor alone lead to an accumulation of cells in G_1 and a reduction of cell number in S-phase (Fig. 2B). Doxorubicin causes arrest in G_1 and G_2/M at the expense of S-phase. The pattern observed with DOX is enhanced upon pretreatment with Pen–ELP–H1, where the G_1 fraction increases slightly as compared to treatment with DOX alone (Fig. 2B). Treatment of MCF-7 cells with etoposide causes accumulation in both G_1 and G_2/M (Fig. 2C). Adding Pen–ELP–H1 before etoposide treatment slightly enhances the etoposide-induced accumulation in G_1, but has no significant effect on the G_2/M accumulation (Fig. 2C).

3.3. *Doxorubicin uptake and efflux*

In order to determine whether the enhanced toxicity of DOX was due to an increase in the cellular accumulation of drug after Pen–ELP–H1 treatment, intracellular DOX levels were assessed by flow cytometry using the intrinsic DOX fluorescence. Doxorubicin uptake by MCF-7 cells was measured by exposing cells to DOX (1 μM) for different lengths of time with or without pretreatment with Pen–ELP–H1. The amount of DOX in the cells increased with increasing exposure time, but pretreatment with Pen–ELP–H1 had no effect on the DOX levels (Fig. 3A).

Although DOX accumulation in cells was unaffected by polypeptide pretreatment, it is still possible that the enhanced cytotoxicity may be the result of Pen–ELP–H1 inhibition of DOX efflux from the cells. In order to test this, cells were loaded with DOX (1 μM) for 5 h, the DOX containing media was replaced with fresh media, and cells were harvested at varying time points after DOX removal for flow cytometry analysis. Fig. 3B shows that some DOX did diffuse from MCF-7 cells after the drug was removed. However, pretreatment of cells with Pen–ELP–H1 had no effect on the rate at which the DOX efflux occurred. Since Pen–ELP–H1 is not modulating the DOX IC_{50} by affecting the flux of drug into or out of the cells, the mechanism is likely due to the effect of the c-Myc inhibitory polypeptide on the transcription levels of c-Myc target genes.

3.4. *Mechanism for enhanced drug toxicity*

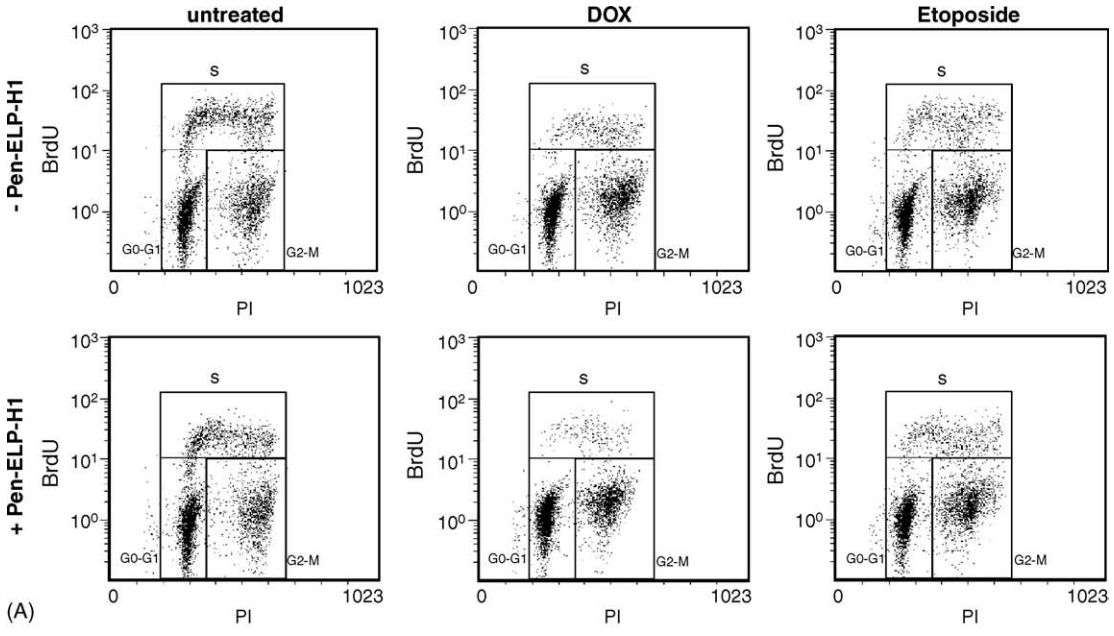
As a transcription factor, c-Myc influences the transcription levels of a large variety of genes. One of the genes under c-Myc control encodes the enzyme ornithine decarboxylase [24]. ODC catalyzes the first and rate-limiting step in the synthesis of cellular polyamines [25]. Many previous studies have indicated that polyamine levels may influence cell proliferation, carcinogenesis and apoptosis (reviewed in Ref. [26]). In addition, a potent irreversible inhibitor of ODC, α-difluoromethylornithine, has been shown to enhance the toxicity of many chemotherapeutic drugs, including doxorubicin and cisplatin [27,28]. In a previous study, it was shown that interruption of the c-Myc/Max heterodimer by the Pen–ELP–H1 polypeptide causes a reduction in the steady state levels of ODC mRNA to less than 40% of control levels [7]. This observation led to the hypothesis that modulation of doxorubicin and etoposide toxicity may be due to a reduction in the amount of ODC in the cell. In order to test this hypothesis, a combination of pretreatment with the ODC inhibitor DFMO and/or Pen–ELP–H1 was used in the doxorubicin and etoposide toxicity assay. MCF-7 cells were pretreated with Pen–ELP–H1 (20 μM), DFMO (50 μM) or both before a 72 h exposure to DOX (750 nM) or etoposide (200 μM). Both DFMO and Pen–ELP–H1 enhanced the DOX-induced cell killing by similar amounts (Fig. 4A). Pretreatment with the combination of Pen–ELP–H1 and DFMO showed no greater enhancement of DOX toxicity than either agent used alone (Fig. 4A). Similar results were obtained with Pen–ELP–H1 and/or DFMO combined with etoposide (Fig. 4B). The fact that Pen–ELP–H1 and DFMO do not act additively or synergistically implies that both agents are affecting the same pathway, and either agent alone is sufficient to completely inhibit this pathway.

3.5. *Cell line dependence*

In order to establish whether the effect of Pen–ELP–H1 pretreatment is specific to MCF-7 cells, the doxorubicin and etoposide IC_{50} assay was repeated on two other cell lines, HeLa cervical carcinoma cells and MES-SA uterine sarcoma cells. As shown in Table 2, Pen–ELP–H1 pretreatment reduced the DOX IC_{50} by 1.5-fold in HeLa cells. Also, the etoposide IC_{50} was reduced 1.4-fold. Similar results were seen in MES-SA cells, where the DOX IC_{50} was reduced 2-fold, and the etoposide IC_{50} was reduced 1.8-fold. This data demonstrates that the ability of Pen–ELP–H1 to modulate the toxicity of topoisomerase II inhibitors is not specific to MCF-7 cells, but seems to be a general property of inhibition of c-Myc transcriptional activation.

4. Discussion

The non-specific side effects of chemotherapy necessitate the improvement of drug delivery methods and the development



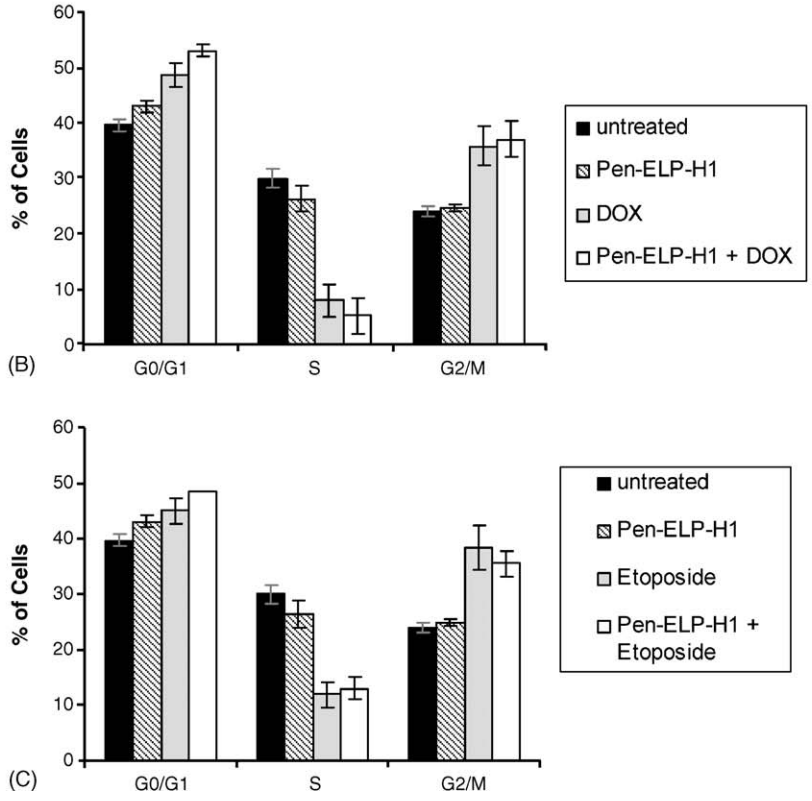


Fig. 2 – Cell cycle analysis. Cells were pretreated with 20 μM Pen–ELP–H1 on day 1 and 100 nM DOX or 1 μM etoposide on day 4 and analyzed on day 5 by flow cytometry with BrdU and propidium iodide staining. Raw data for a single representative experiment is shown (A), and four independent experiments with DOX (B) and etoposide (C) were averaged. Percentages were determined by analysis of 5000 cells/sample, and results shown represent the mean ± S.E.M.

of approaches to increase a drug's therapeutic index. In this work, a polypeptide inhibitor of c-Myc was shown to enhance the antiproliferative effect caused by doxorubicin and etoposide. The effect was specific to topoisomerase II inhibitors, as no modulation of camptothecin or cisplatin was observed. Also of note is the lack of a large inhibition in cell proliferation by the Pen–ELP–H1 agent alone (as seen by the difference between the control and pretreated curves at zero drug concentration in Fig. 1). This is consistent with data collected in the previous study of Pen–ELP–H1, in which the polypeptide was shown to reduce the doubling time of the MCF-7 cells, requiring 11 days to observe significant inhibition of cell

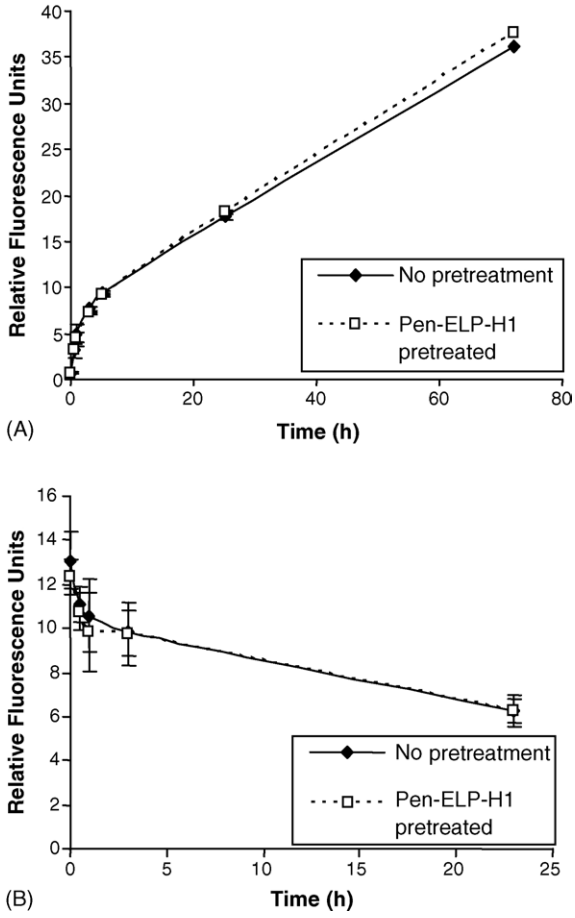


Fig. 3 – DOX accumulation and efflux. (A) DOX accumulation. Cells were exposed to DOX (1 μM) for various lengths of time with or without Pen–ELP–H1 pretreatment. (B) DOX efflux. Cells (Pen–ELP–H1 pretreated and control) were exposed to 1 μM Dox for 5 h, then rinsed and placed in fresh media and analyzed at the indicated time points. DOX levels were determined using the intrinsic DOX fluorescence measured by flow cytometry. Data represent the mean ± S.E.M. of three independent experiments (n = 5000 cells).

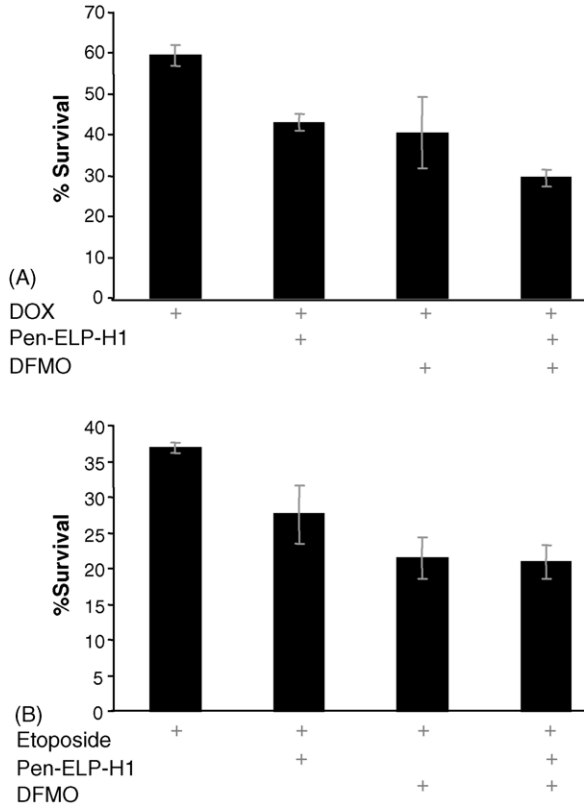


Fig. 4 – Proliferation with DFMO. Cells were pretreated with 20 μM Pen–ELP–H1 or 50 μM DFMO on day 1, and treated with 750 nM DOX (A) or 200 μM etoposide (B) on day 4. Cell viability was determined on day 7 using the MTS cell viability assay. Results represent the mean ± S.E.M. of three independent experiments.

numbers [7]. Also, the order in which the agents were added was important (data not shown). Pen–ELP–H1 must be added before the topoisomerase II inhibitor in order to enhance its toxicity, which suggests that Pen–ELP–H1 induces a downstream effect that is important for drug toxicity.

In order to demonstrate whether DOX modulation by Pen–ELP–H1 is specific to MCF-7 cells, two other cancer cell lines were tested. The c-Myc inhibitory polypeptide enhanced DOX and etoposide toxicity in both MES-SA and HeLa cells. This demonstrates that the ability of the H1 peptide to

Table 2 – Effects of Pen–ELP–H1 pretreatment on IC_{50} values in HeLa and MES-SA Cells

Cell line	Drug	IC_{50} (mean ± S.E.M.)	IC_{50} after Pen–ELP–H1 pretreatment (mean ± S.E.M.)
HeLa	Doxorubicin[a] (nM)	13.9 ± 1.3	9.4 ± 1.2
	Etoposide[a] (nM)	630.5 ± 59.2	464.6 ± 45.4
MES-SA	Doxorubicin[a] (nM)	33.7 ± 7.1	16.3 ± 1.6
	Etoposide[a] (μM)	17.3 ± 3.1	9.8 ± 3.1

IC_{50} values were collected for HeLa and MES-SA cells. The DOX and etoposide IC_{50} was determined with and without Pen–ELP–H1 pretreatment. Curves were fit using an exponential equation, and the IC_{50} value shown represents the mean ± S.E.M. of at least five independent determinations each performed with four replicates per drug concentration. Statistical significance was assessed using an unpaired Student's t-test.

[a] Difference between control and Pen–ELP–H1 pretreated IC_{50} is statistically significant as determined by an unpaired Student's t-test ($P < 0.05$).

enhance therapy with topoisomerase II inhibitors may be generally applied.

Pretreatment of MCF-7 cells with Pen–ELP–H1 caused an accumulation in the G_0/G_1 phase of the cell cycle. It is possible that the enhanced drug toxicity seen after Pen–ELP–H1 treatment is due to enhanced sensitivity of cells in G_0/G_1. However, this explanation is unlikely since previous studies have reported that cells arrested in G2 are more sensitive to topoisomerase II inhibitors (discussed in Ref. [29]) than cells in G_0/G_1. Even if cell cycle modulation does not explain the mechanism by which Pen–ELP–H1 enhances drug toxicity, pretreatment of cells with the c-Myc inhibitory polypeptide does augment the DOX and etoposide-induced accumulation in G_1 phase of the cell cycle.

In an attempt to elucidate the mechanism by which Pen–ELP–H1 modulates drug toxicity, the cellular levels of DOX were analyzed with and without polypeptide pretreatment. Pen–ELP–H1 had no affect on the uptake or efflux of DOX, showing clearly that the enhanced drug toxicity cannot be explained simply by alterations of cellular drug levels. A more likely explanation is that a c-Myc controlled gene that has a role in drug toxicity is affected by inhibition of c-Myc/Max heterodimerization. One such c-Myc regulated gene is ODC [24], which catalyzes the first step in polyamine biosynthesis [25]. Inhibition of ODC has been shown to increase doxorubicin and etoposide toxicity in several cell lines [27,28,30], and the effect is attributed to lowering the cellular polyamine levels. Lowering polyamine levels is hypothesized to influence topoisomerase II binding and cleavage by affecting chromatin structure [28,31–33]. Our previous work showed that Pen–ELP–H1 is capable of lowering ODC mRNA levels [7]. In the current study, inhibition of ODC using DFMO produced a similar enhancement of DOX and etoposide toxicity as did pretreatment with Pen–ELP–H1. However, the agents did not act additively or synergistically. This result may be explained if both agents are acting on the same pathway, and both agents are capable of producing complete inhibition of ODC activity. After reduction in ODC levels by treatment with Pen–ELP–H1 [7], the likely mechanism involves a reduction in polyamine levels and enhanced sensitivity to topoisomerase II inhibitors. While this mechanism is very probable, we cannot rule out the possibility that other c-Myc/Max controlled genes may also be involved in modulating drug toxicity.

Previous in vivo studies have used DFMO to inhibit ODC activity, and doxorubicin has been used successfully in combination with DFMO to prolong the survival of rats carrying prostatic adenocarcinoma [30]. However, cell response to DFMO in combination with chemotherapeutic drugs is complex and depends on the cell type, drug, DFMO concentration and treatment schedule [27,31,34,35]. Another limitation of DFMO combination therapy is inherent tumor resistance or developed resistance following DFMO exposure (reviewed in Ref. [27]). Since Pen–ELP–H1 is known to reduce ODC levels, and effective augmentation of DOX and etoposide was observed in all three cell lines tested, this approach represents a promising alternative to DFMO for combination therapy.

Inhibiting c-Myc function has most commonly been approached by the use of c-Myc directed antisense oligonucleotides [36]. Lowering c-Myc levels using an antisense strategy has been shown to increase cisplatin toxicity [37–39], but c-Myc antisense therapy had no effect on doxorubicin sensitivity in small cell lung carcinoma cell lines [37,40]. This discrepancy may be explained simply by the use of different cell types, or there may be an inherent difference in the effects of c-Myc knockdown versus inhibition of c-Myc and Max dimerization. It is clear from our results that, in the cell lines tested, simply blocking the c-Myc/Max interaction is sufficient to augment DOX and etoposide toxicity. When applied in a clinical setting, antisense oligonucleotide therapy often faces delivery problems and off-target toxicity [41], which may be overcome by the use of a polypeptide-based molecule. This advantage, combined with the fact that the H1 peptide produced differing results from antisense oligonucleotides when combined with DOX, suggests that Pen–ELP–H1 may be useful in applications where c-Myc antisense approaches fail.

This represents, to our knowledge, the first demonstration that a molecule capable of inhibiting the c-Myc/Max interaction can effectively improve the cytotoxicity of topoisomerase II inhibitors. The approach applied here is advantageous because it employs a polymeric carrier to deliver the inhibitory polypeptide, which offers improved pharmacokinetics and the possibility of tumor targeting. ELP-based inhibitory peptides are also attractive because they are easy to modify at the sequence level [42], expressed at high yield in *E. coli* [20], and easily purified by simple thermal cycling [43].

Pen–ELP–H1 is a novel polypeptide that combines the advantages of a macromolecular carrier with a new generation of inhibitory molecule. Because Pen–ELP–H1 enhances sensitivity of the tumor cells to topoisomerase II inhibitors, it shows promise in its ability to improve doxorubicin and etoposide therapy. Overexpression of c-Myc is a common event in carcinogenesis, and its aberrant expression enhances the growth and proliferation of the cancerous cells [4]. The use of a c-Myc inhibitor will therefore affect the cancerous cells more significantly than the normal tissue. This fact, combined with the increase in DOX and etoposide toxicity, means that these drugs could be administered systemically at a lower dose resulting in minimized side effects. This approach offers an alternative to vector-delivered drugs and holds great potential in improving future chemotherapy treatments.

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