Anti-Cancer Drugs 1999, **10**, pp. 47–54

Preclinical paper

The cardioprotective and DNA topoisomerase II inhibitory agent dexrazoxane (ICRF-187) antagonizes camptothecin-mediated growth inhibition of Chinese hamster ovary cells by inhibition of DNA synthesis

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Dexrazoxane (ICRF-187), which is clinically used to reduce doxorubicin-induced cardiotoxicity, has cell growth inhibitory properties through its ability to inhibit the catalytic activity of DNA topoisomerase II. A study was undertaken to investigate whether preincubating Chinese hamster ovary cells (CHO) with dexrazoxane prior to camptothecin treatment resulted in potentiation. Camptothecin is a DNA topoisomerase I poison. It was found that pretreating CHO cells with concentrations of dexrazoxane sufficient to strongly inhibit topoisomerase II for periods from 18 to 96 h resulted in significant antagonism of camptothecin-mediated growth inhibition. Lower concentrations that were sufficient to cause partial inhibition of topoisomerase II and partial dexrazoxane-mediated cell growth inhibition had little effect on camptothecin-mediated growth inhibition. Neither topoisomerase I protein levels nor camptothecin-induced topoisomerase I–DNA covalent complexes were affected by dexrazoxane concentrations that were sufficient to cause antagonism of camptothecin-induced growth inhibition. However, under these experimental conditions, dexrazoxane caused a decrease in DNA synthesis. Therefore, results presented here confirm the importance of the DNA synthesis-dependent replication fork interaction with topoisomerase I–DNA covalent complexes for the expression of camptothecin activity. It is concluded that dexrazoxane and camptothecin analogs should be used with caution in combination chemotherapy. [© 1999 Lippincott Williams & Wilkins.]

Key words: Bisdioxopiperazine, camptothecin, cytotoxicity, CHO, dexrazoxane, DNA, ICRF-187, topoisomerase II.

This work was a joint and equal effort from both laboratories. This work was supported in part by the Medical Research Council of Canada, and NIH grants CA77468 and CA74972.

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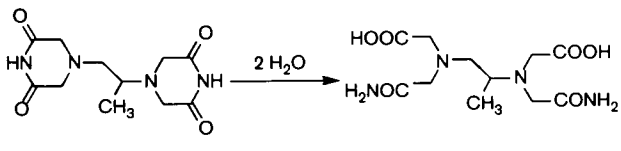
Introduction

Dexrazoxane (ICRF-187, Zinecard®; Figure 1a) is in clinical use in the US and Canada, where it is being used to reduce doxorubicin-induced cardiotoxicity.[1-3] Dexrazoxane likely exerts its cardioprotective effects through its rings-opened hydrolysis product ADR-925 (Figure 1a) by virtue of its ability to strongly chelate free iron,[4] or to quickly and efficiently remove iron from its complex with doxorubicin,[5] thus reducing doxorubicin-induced iron-mediated oxygen free radical damage.[6]

Dexrazoxane is the (+)-(*S*)-enantiomer of racemic ICRF-159, which was originally developed as an antitumor agent.[7,8] The bisdioxopiperazines [including dexrazoxane, ICRF-159 (razoxane), ICRF-154 and ICRF-193] are now known to be strong inhibitors of mammalian DNA topoisomerase II.[9-13] Topoisomerase II alters DNA topology by catalyzing the passing of an intact DNA double helix through a transient double-stranded break made in a second helix.[14] Topoisomerase I, by contrast, alters DNA topology through a transient single-stranded break.[15,16] The topoisomerase I poison camptothecin,[15,16] and the topoisomerase II poisons such as doxorubicin, etoposide and amsacrine, are thought to inhibit cell growth by virtue of their ability to stabilize a covalent topoisomerase I- or topoisomerase II-DNA intermediate (the cleavable complexes), respectively.[14,17,18] In contrast, dexrazoxane and the bisdioxopiperazines[9,10] inhibit topoisomerase II *in vitro*[9,19] and in cultured cells,[10] without inducing cleavable complex formation. The bisdioxopiperazines can, in fact, reduce protein-DNA cross-links induced by etoposide, amsa-

(a)



Dexrazoxane (ICRF-187) ADR-925

(b)

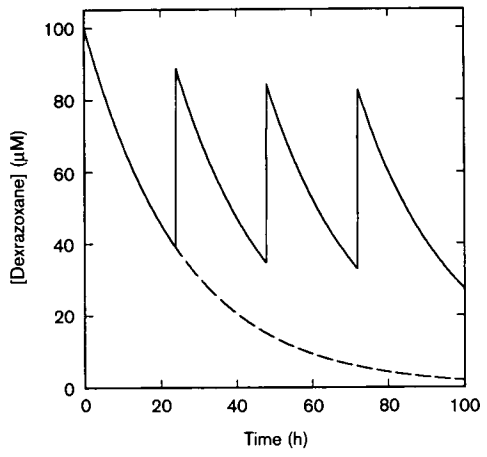


Figure 1. (a) Reaction scheme for the hydrolysis of dexrazoxane to ADR-925. (b) Calculated concentrations of dexrazoxane present in cell culture medium under the conditions used in this study. The continuous solid line is calculated assuming that the initial dexrazoxane concentration of 100 μM has had additional dexrazoxane (50 μM) added to the medium at 24, 48 and 72 h. The broken line, which is a continuation of the solid line from 0 to 24 h, is the concentration of dexrazoxane in the medium that would be obtained if additional dexrazoxane had not been added to the medium. The concentrations were calculated assuming that dexrazoxane is lost from the culture medium with a first-order rate constant of 0.039 h^{-1} ($t_{1/2}$=18 h) under conditions identical to those used here.[13]

crine, daunorubicin and doxorubicin,[9,10,19] and antagonize the growth inhibitory effects of doxorubicin and daunorubicin.[12,19] The bisdioxopiperazines may act by trapping the enzyme in the form of a closed protein clamp,[20] thus preventing the formation or stabilization of the topoisomerase II-DNA covalent intermediate.

A preliminary report[21] suggested that pretreatment of human leukemia K562 cells with dexrazoxane potentiated the growth inhibitory effects of the camptothecin analog topotecan in a sequence- and schedule-dependent manner. It has been previously demonstrated[22] that topotecan increased topoisomerase IIα levels resulting in an increased sensitivity of human tumor xenografts to etoposide. Also, in yeast the absence of topoisomerase I can be compensated for by the presence of topoisomerase II.[23,24] In an analogous manner, it was hypothesized that inhibition of topoisomerase II might result in a compensatory increase in topoisomerase I, making the cells more sensitive to camptothecin. Increased levels of topoisomerase I have been shown to result in an increased sensitivity to camptothecin.[23,24] Thus, a study was undertaken to clarify if, and if so under what conditions, the sequential use of the topoisomerase II catalytic inhibitor dexrazoxane and the topoisomerase I poison camptothecin could result in potentiation. Also, given the widening use of dexrazoxane as a cardioprotective agent, it is important to determine whether dexrazoxane has any effect on camptothecin-mediated growth inhibition, as dexrazoxane is likely to be used in combination with camptothecin analogs in the future during the course of multi-drug therapy.

Materials and methods

Cell culture and growth inhibition assays

Chinese hamster ovary (CHO) cells (type AA8; ATCC CRL-1859), obtained from the ATCC (Rockville, MD), were grown in α-minimum essential medium (α-MEM) (Gibco/BRL, Burlington, Canada) containing 20 mM HEPES (Sigma, St Louis, MO), 100 U/ml penicillin G, 100 μg/ml streptomycin, 10% fetal calf serum (Gibco/BRL) in an atmosphere of 5% CO_2 and 95% air at 37°C (pH 7.4) as previously described.[12] Cells in exponential growth were harvested and seeded (2000 cells/well) in 96-well microtiter plates (100 μl/well) and allowed to attach for 24 h or as indicated. Dexrazoxane (Pharmacia & Upjohn, Columbus, OH), was dissolved in α-MEM and was added at the times indicated to give a final volume of 200 μl/well. All growth inhibitory experiments were conducted with continuous exposure to drugs for the times indicated. The cell growth was determined using a 96-well microtiter plate by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay as previously described.[13] Typically six replicates were measured at each drug concentration. The IC_{50} values for growth inhibition were obtained from a non-linear least-squares fit of the absorbance–drug concentration data to a three- or four-parameter logistic equation (SigmaPlot; Jandel, San Rafael, CA) as appropriate. Unless otherwise indicated, the errors quoted are SEMs, or in regressions linear or non-linear estimates of the SEM.

Western blot analysis of topoisomerase I

Whole cell lysates were prepared from 2.5×10^6 CHO cells by dissolving cell pellets in 2 × SDS-PAGE sample buffer (100 mM Tris-HCl, pH 6.8, 2% SDS, 20% glycerol and 1.0% β-mercaptoethanol). Lysates were sonicated, boiled for 5 min and the protein content was determined by the BioRad (Hercules, CA) protein assay. Protein (20 μg/well) was loaded onto 7% SDS-PAGE gels. Resolved proteins were electrophoretically transferred to nitrocellulose and incubated with rabbit polyclonal antisera to human topoisomerase I (Topo-Gen, Columbus, OH). Bound antibody was detected using a Renaissance chemiluminescence kit (NEN, Life Science Products, Boston, MA). Autoradiographic signals were quantified by densitometric scanning using a Molecular Dynamics densitometer (Molecular Dynamics, Sunnyvale, CA).

Topoisomerase I–DNA covalent complexes

Topoisomerase I-DNA covalent complex formation in intact cells was measured as previously described for topoisomerase II-DNA complexes.[25] Early-log growth CHO cells were labeled for 48 h with 0.5 μCi/ml [^{3}H]dThd ([*methyl*-^{3}H]thymidine) (0.5 Ci/mmol; NEN, Life Sciences Products) and 0.1 μCi/ml [^{14}C]leucine (318 mCi/mmol) in α-MEM containing 20 mM HEPES (Sigma), 100 U/ml penicillin G, 100 μg/ml streptomycin and 10% fetal calf serum. For the last 24 h of this incubation dexrazoxane was added to a final concentration of 100 μM. Cells were then washed and resuspended in Buffer 1 (pH 7.4) consisting of 115 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM NaH_2PO_4, 25 mM HEPES and 10 mM glucose at 37°C at a final cell number of approximately 1.0×10^6 cells/well in a 24-well cell culture plate (Costar, Cambridge, MA) for experimentation. Cells were then treated for 1 h with 0-100 μM camptothecin at 37°C. Reactions were stopped by aspiration of buffer and washing cells twice with 2.5 ml ice-cold PBS. Cells were then removed from plates by addition of trypsin. Cells were then pelleted and washed, lysed, cellular DNA sheared, and protein-DNA complexes precipitated with SDS and KCl as described.[25] Topoisomerase I-DNA covalent complexes were quantified by scintillation counting and [^{3}H]DNA was normalized to cell number using the co-precipitated ^{14}C-labeled protein as an internal control.

DNA synthesis

CHO cells ($1\text{-}2 \times 10^7$) were plated in 175 cm^2 cell culture flasks. After 2-3 h attachment of cells, dexrazoxane (100 μM) was added and the cells were incubated for an additional 24 h. Trypsin-harvested cells were washed first in α-MEM containing 10% fetal calf serum, then resuspended at a concentration of 1×10^7 cells/ml in Buffer 1 at 37°C. [^{3}H]dThd was added to the control and drug-treated cell suspensions at a final concentration of 10 μM and a final specific activity of 3 μCi/ml. Samples (0.5 ml) were taken immediately after addition of [^{3}H]dThd (zero time controls) and every 4 min thereafter, and added onto 400 μl silicone oil (Nyosil 50; William F Nye, Speciality Lubricants, New Bedford, MA) which was pre-layered on top of 300 μl of 10% ice-cold trichloroacetic acid (TCA) in 1.5 ml microcentrifuge tubes. Samples were spun immediately for 10 s in a microcentrifuge at room temperature. Pelleted cells were kept on ice until the entire 40 min incubation period was completed. After incubation, the extracellular buffer layer was aspirated and the top of the oil layer was washed twice with ice-cold Buffer 1. The oil layer was then carefully aspirated, and the remaining TCA layer was vortexed vigorously and set on ice for 10 min. Cells were pelleted at 10 000 r.p.m. for 10 min at 4°C in a microcentrifuge and washed with 500 μl of ice-cold 10% TCA two more times. After the final wash, TCA was aspirated carefully so that only the pellets remained. Pellets were dissolved in 300 μl 1 M KOH at 65°C for 10 min and then neutralized with 300 μl of 1 N HCl. Each sample (500 μl) was added to glass vials containing 3.5 ml of scintillation fluid (Ecolyte; ICN Biomedicals, Irvine, CA) and radioactivity was assessed by liquid scintillation spectrometry.

Results

Effect of preincubation of cells with dexrazoxane on camptothecin-mediated growth inhibition

Dexrazoxane, which is the only active topoisomerase II inhibitory form of the drug, is hydrolyzed to ring-opened inactive forms with a half-life of 18 h[13] under the conditions used in this study (Figure 1b). Figure 1b shows the calculated dexrazoxane concentration in the culture medium when dexrazoxane is replenished every 24 h at one-half its initial concentration. This dosing protocol gives an approximately constant level of dexrazoxane exposure to the cells,[13] similar to that

previously reported.[21] The doubling time of the CHO cells used in this study is 12 h (data not shown) and, thus, topoisomerase II would be strongly inhibited over its whole cell cycle at high dexrazoxane concentrations. It was previously reported[21] that relatively long exposures (96 h) to K562 cells at low, nearly constant, concentrations of dexrazoxane (0.7-3.6 μM) results in a 25-fold potentiation of topotecan-mediated growth inhibition.

As shown in Figure 2(a) the effect of preincubating cells with either 1 or 2 μM dexrazoxane for 18 h and then exposing them to camptothecin for 72 h (with dexrazoxane being replenished every 24 h at one-half the initial concentration) resulted in little change in the IC_{50}. For camptothecin alone the IC_{50} was 0.024 ± 0.007 μM, which compares to 0.025 ± 0.008 and 0.035 ± 0.015 μM with the addition of 1 and 2 μM dexrazoxane, respectively. The IC_{50} for inhibition of topoisomerase II by dexrazoxane has been determined to be 13 μM.[11,13] Thus, it would be expected that the concentrations of dexrazoxane used in the experiments described in Figure 2(a) would result in only partial inhibition of topoisomerase II (and partial dexrazoxane-mediated cell growth inhibition). As shown in Figure 2(b) experiments were also conducted at high concentrations of dexrazoxane, where nearly total inhibition of topoisomerase II would be occurring. These results show that a high degree of antagonism of camptothecin-mediated growth inhibition occurred when the cells were preincubated with 100 μM dexrazoxane for 18 h (and then replenished every 24 h at one-half the initial dexrazoxane concentration). A nearly 50-fold increase in IC_{50} was observed, from 0.0118 ± 0.004 in the absence of dexrazoxane, to 0.58 ± 0.14 μM in its presence. The IC_{50} for dexrazoxane alone was also determined to be 1.7 ± 0.02 μM, which compares to a previously determined value of 1.8 μM.[26] It is a characteristic of dexrazoxane-mediated growth inhibition using the MTT assay that a biphasic drug-response curve is seen with a plateau region in the 20-1000 μM dexrazoxane range.[11,26]

Another experiment (data not shown) was also carried out whereby the cells were exposed to various concentrations of dexrazoxane for 96 h in a T-flask (with replacement of the original concentration of dexrazoxane every 24 h), trypsinized, replated and allowed to attach for 24 h, and then exposed to various concentrations of camptothecin for 72 h, while maintaining exposure to dexrazoxane by replenishment of dexrazoxane every 24 h at one-half the initial dexrazoxane concentration (except at 100 μM dexrazoxane). With this drug treatment protocol, which is for the same time as that used by Synold,[21] it was found that the IC_{50} for camptothecin-mediated growth inhibition maximally increased 24-

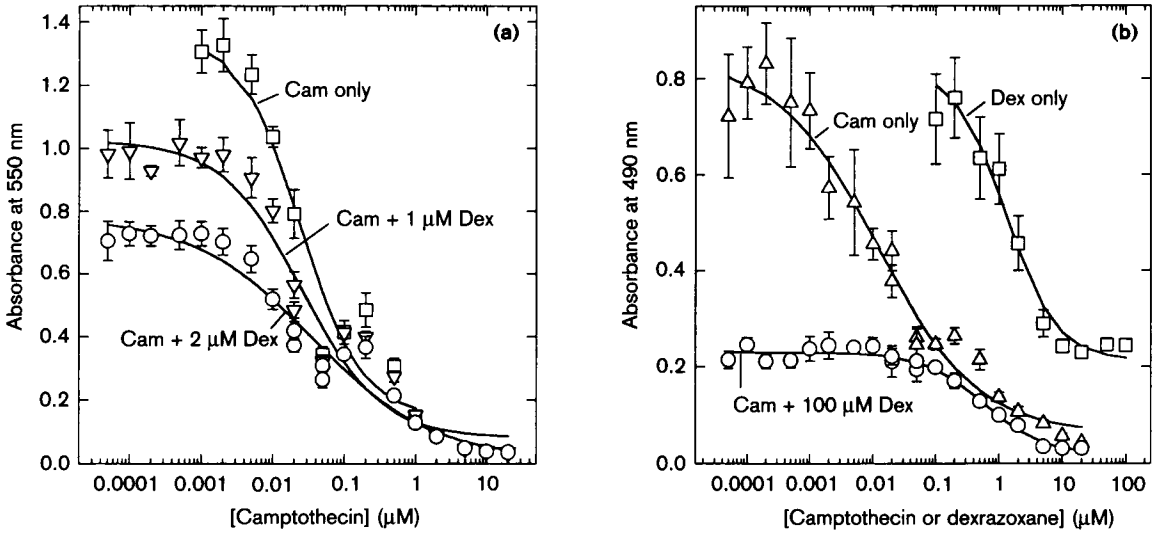


Figure 2. (a) Camptothecin (Cam)-mediated growth inhibition of CHO cells in the presence of 0 (□), 1 (▽) or 2 (○) μM dexrazoxane (Dex) measured using an MTT assay. In this experiment the cells were initially preincubated with either 1 or 2 μM dexrazoxane for 18 h and then replenished with 0.5 or 1 μM dexrazoxane, respectively, at 24 h intervals thereafter. The cells were dosed with varying concentrations of camptothecin at 18 h and then allowed to grow for a further 72 h before the MTT assay. (b) Conditions are as in (a), except that the cells were initially dosed with 100 μM (○) dexrazoxane and replenished with 50 μM dexrazoxane every 24 h thereafter. Also shown are the growth inhibitory effects of dexrazoxane alone (□). The solid lines are calculated from non-linear least-squares fits of the data to a logistic equation. The error bars shown are SDs.

fold. IC_{50}s of 0.022 ± 0.004, 0.025 ± 0.006, 0.035 ± 0.010, 0.032 ± 0.006 and 0.53 ± 0.25 μM were observed in the presence of 0, 0.2, 0.5, 2 and 100 μM dexrazoxane, respectively. This result indicates that prolonged exposure to a wide range of dexrazoxane concentrations does not result in potentiation of camptothecin-mediated growth inhibition, but rather a high degree of antagonism at high dexrazoxane concentration.

Experiments were also carried out using shorter camptothecin exposure times in an attempt to model the camptothecin exposure that a tumor might receive *in vivo*. As shown in Figure 3, the IC_{50}s decreased only marginally when cells were preincubated with a range (5-200 μM) of dexrazoxane concentrations for 26 h. IC_{50}s of 2.2 ± 0.4, 1.5 ± 0.1, 0.8 ± 0.6, 0.9 ± 0.09 and 1.1 ± 0.1 μM were observed after preincubation with 0, 5, 30, 50, 100 and 200 μM dexrazoxane, respectively. In these experiments cells were plated for 24 h, after which dexrazoxane was added for a further 24 h before camptothecin was added. Dexrazoxane was not replenished or washed off in these experiments. These results indicate that the sensitivity of camptothecin-mediated growth inhibition is severely attenuated when the time the cells are exposed to camptothecin is reduced.

In order to better understand the mechanism(s) by which dexrazoxane antagonizes camptothecin action, the level of topoisomerase I was examined in CHO cells incubated for 18-66 h with 100 μM dexrazoxane.

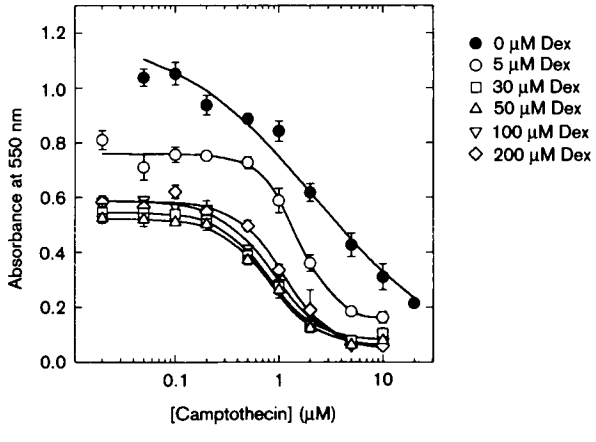


Figure 3. (a) Camptothecin-mediated growth inhibition of CHO cells in the presence of 0 (●), 5 (○), 30 (□), 50 (△), 100 (▽) or 200 (◇) μM dexrazoxane. In these experiments the cells were attached for 24 h, dosed with the concentrations of dexrazoxane indicated for 26 h, and then dosed with varying concentrations of camptothecin and then allowed to grow for a further 48 h before the MTT assay. The solid lines are calculated from a non-linear least-squares fits of the data to a logistic equation. The error bars shown are SDs.

As shown in Figure 4, dexrazoxane did not affect topoisomerase I protein expression in CHO cells. When CHO cells were pre-treated with 100 μM dexrazoxane for 24 h, it was found that there was

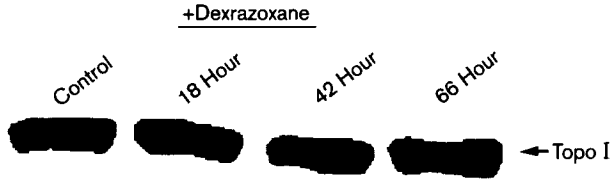


Figure 4. Topoisomerase I protein levels in CHO cells treated with 100 μM dexrazoxane for various time periods. Dexrazoxane (100 μM) was added to cell monolayers 6 h after plating CHO cells at a density of 10000 cells/cm^2. Dexrazoxane concentration was replenished 18 and 42 h later by addition of one-half the initial concentration of dexrazoxane (50 μM). Samples were taken 18, 42 and 66 h after initial addition of dexrazoxane, and processed for Western blot analysis of topoisomerase I protein levels as described in Material and methods.

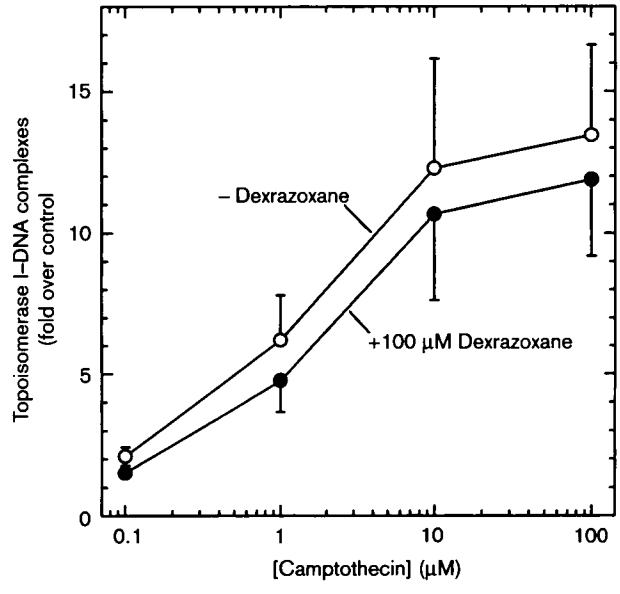


Figure 5. Formation of camptothecin-induced topoisomerase I–DNA covalent complexes in CHO cells (○) and CHO cells pretreated for 24 h with 100 μM dexrazoxane (●). Cells were prelabeled with [^{3}H]dThd and [^{14}C]leucine. Cells were then incubated for 1 h in the presence of various concentrations of camptothecin. KCl–SDS precipitable complexes were isolated, and the ^{3}H counts were normalized using ^{14}C as an internal standard for cell number as described in Material and methods. Results are expressed as fold-increase in camptothecin-induced topoisomerase I–DNA complexes relative to complexes isolated from cells incubated in the absence of camptothecin. Data points shown are the means from four separate experiments performed on separate days.

no change in the ability of camptothecin (0.1-100 μM) to induce topoisomerase I-DNA covalent complexes compared to CHO cells not treated with dexrazoxane (Figure 5). Hence, dexrazoxane antagonism of camptothecin activity is not due to a reduction in the intracellular target for camptothecin or to a decrease in topoisomerase I-DNA protein complexes. A 24 h incubation of CHO cells with 100 μM dexrazoxane resulted in a 2-fold reduction in DNA synthesis as measured by thymidine incorporation into TCA precipitates) (Figure 6). These results suggest that dexrazoxane antagonizes camptothecin activity by reducing DNA synthesis-dependent replication fork collisions with topoisomerase I-DNA covalent complexes. The cytotoxicity of topoisomerase I poisons have previously been shown to be dependent on replication fork collisions with topoisomerase I-DNA covalent complexes.[27-29]

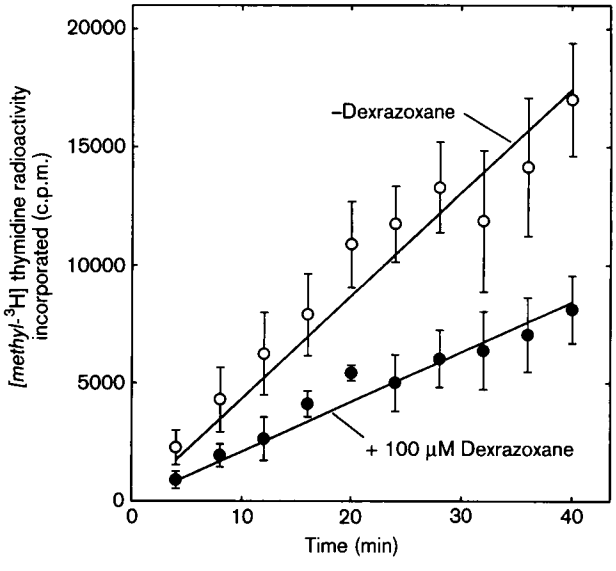


Figure 6. Time course of [^{3}H]dThd incorporation in TCA precipitates from CHO cells (○) or CHO cells treated for 24 h with 100 μM dexrazoxane (●). At the specified times after addition of 10 μM [^{3}H]dThd, portions of the cell suspensions (5×10^6 cells) were centrifuged through silicone oil into 10% TCA. The TCA precipitate was analyzed for radioactivity as described in Materials and methods. Data points shown are the mean ± SEM from four separate paired experiments performed on separate days. By linear regression analysis from the slopes of the plots the [^{3}H]dThd incorporation rates were found to be 374 ± 32 and 190 ± 13 c.p.m./min for CHO cells and dexrazoxane-treated CHO cells, respectively. Analyzing the rates for [^{3}H]dThd incorporation for each of the four paired experiments indicated that there was a statistically significant decrease in the rate of DNA synthesis in cells treated with dexrazoxane ($p < 0.001$ by *t*-test for the comparison of the slopes of two regression lines).

Discussion

This study has shown that if cells are preincubated with a high concentration of dexrazoxane for various times before treatment with camptothecin, either significant antagonism results (up to a 50-fold increase in IC_{50}) with a 72 h camptothecin treatment protocol) or little more than additivity occurs with a 48 h camptothecin treatment protocol. Thus, this study is unable to confirm the preliminary findings of significant potentiation reported by Synold[21] using the camptothecin analog topotecan. Topotecan is structurally very similar to camptothecin and both are known to be topoisomerase I inhibitors.[15] Thus, it is unlikely that the difference in the results found here and by Synold[21] are due to differences in the two drugs used, though that cannot be absolutely ruled out.

The most significant effect of dexrazoxane on decreasing the growth inhibitory effects of camptothecin was at the higher (100 μM) doses of dexrazoxane where the cells were exposed to camptothecin for 72 h. At lower dexrazoxane concentrations, where only partial inhibition of topoisomerase II should occur (IC_{50} of 13 μM for inhibition of topoisomerase II by dexrazoxane[11,13]), little effect on the growth inhibitory IC_{50} was observed. Thus, it can be concluded that for significant antagonism of camptothecin-mediated growth inhibition, a high degree of inhibition of topoisomerase II must occur.

Kizaki and Onishi[30] demonstrated that the dexrazoxane analog ICRF-154 had no effect on the expression of topoisomerase I in thymocytes using a reverse transcription-polymerase chain reaction assay. Similarly, results presented here indicated that dexrazoxane had no effect on topoisomerase I protein expression in CHO cells (Figure 4). Hence, dexrazoxane-mediated antagonism of camptothecin activity was not due to a decrease in topoisomerase I levels. The results in Figures 5 and 6 indicated that dexrazoxane inhibited DNA synthesis with no effect on camptothecin-induced topoisomerase I-DNA covalent complexes. These results are consistent with previous reports of decreased camptothecin-induced toxicity correlated with aphidicolin- or hydroxyurea-mediated inhibition of DNA synthesis.[27,28] In these reports DNA synthesis inhibition had no effect on the frequency of DNA strand breakage suggesting that active replication fork interaction with topoisomerase I-DNA covalent complexes is a critical determinant for camptothecin growth inhibition and toxicity.[27,28] Subsequent reports have supported the concept that camptothecin-induced DNA single-strand breaks caused by formation of topoisomerase I-DNA covalent complexes are converted to lethal strand breaks by collision with

active DNA replication forks.[29,31,32] Hence, dexrazoxane-mediated antagonism of camptothecin-induced growth inhibition in CHO is due to inhibition of DNA synthesis. ICRF-159 (razoxane, the racemic form of dexrazoxane) and ICRF-193 have both been shown to inhibit DNA synthesis in mouse embryo fibroblasts by about 55% after 23 h of drug exposure.[33] These results are similar to those shown in Figure 6.

Dexrazoxane is a cytostatic rather than cytotoxic agent, as cells dosed with ICRF-159,[34,35] the racemic form of dexrazoxane, do not significantly increase in number, but do continue to increase in size without division. Cells treated with dexrazoxane or other bisdioxopiperazines for times comparable to those used in this study show that cell cycle progression is blocked at G_2/M,[10] which occurs along with the appearance of multinucleation and high ploidy. The polyploidization is thought to occur because the bisdioxopiperazine ICRF-193 blocks late stages of chromosome condensation and segregation.[36] These cell cycle and morphological changes in cells treated with the bisdioxopiperazines are thought to occur due to the stabilization of a closed protein clamp form of topoisomerase II which traps double DNA strands and prevents proper DNA strand passage reactions.[20] The precise mechanisms by which dexrazoxane-mediated perturbations of the cell cycle cause inhibition of DNA synthesis are currently under investigation.

Typically dexrazoxane is clinically dosed at 600 mg/m^2 30 min prior to doxorubicin dosing. Dexrazoxane has an elimination phase $t_{1/2}$ of 4.2 h and yields a peak plasma concentration of 340 μM.[37] The concentrations of dexrazoxane used in this study are well within those found clinically in the plasma. Thus, if this plasma concentration is even partially obtained in the tumor, dexrazoxane may be significantly inhibiting topoisomerase II. The ability of dexrazoxane to antagonize camptothecin growth inhibition, at higher concentrations at least, suggests that some caution should be used when camptothecin analogs and dexrazoxane are used together.

Conclusion

This study has shown that when dexrazoxane, at concentrations where it strongly inhibits topoisomerase II, is preincubated with CHO cells, significant antagonism of camptothecin-mediated growth inhibition can occur. Thus, the clinical use of camptothecin analogs in combination with dexrazoxane should be approached with caution. This study has also shown that neither topoisomerase I protein levels nor camptothecin-induced topoisomerase I-DNA covalent complexes were affected by dexrazoxane concentrations that caused antagonism of camptothecin-mediated growth inhibition. Dexrazoxane did, however, decrease the rate of DNA synthesis.

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(Received 17 September 1998; accepted 22 September 1998)