Anti-Cancer Drugs 1996, **7**, pp. 558–567

Experimental report

The effect of dexrazoxane (ICRF-187) on doxorubicin- and daunorubicin-mediated growth inhibition of Chinese hamster ovary cells

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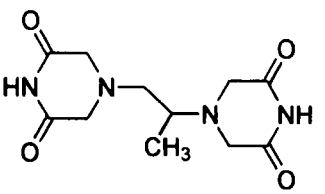
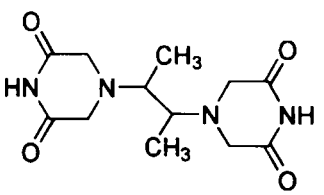
Dexrazoxane (ICRF-187) is clinically used to reduce doxorubicin-induced cardiotoxicity. Because dexrazoxane, doxorubicin and daunorubicin all act on DNA topoisomerase II, a study was undertaken to see what effect dexrazoxane had on the growth inhibitory effects of doxorubicin and daunorubicin towards Chinese hamster ovary cells. Dexrazoxane exhibited significant antagonism of doxorubicin- and daunorubicin-mediated growth inhibition when the cells were preincubated with dexrazoxane before the anthracycline was added. Continuous exposure of cells to either anthracycline and low concentrations of dexrazoxane resulted in additive growth inhibitory effects at low anthracycline concentrations, and no effect at higher anthracycline concentrations.

Key words: antagonism, cytotoxicity, daunorubicin, dexrazoxane, doxorubicin, topoisomerase II.



ICRF-187 (dexrazoxane)



ICRF-193

Figure 1. Structures of the bisdioxopiperazines dexrazoxane (ICRF-187) and ICRF-193 (*meso* form).

Introduction

Dexrazoxane (ICRF-187, Zinecard®, Figure 1) has recently been approved for clinical use in the USA and Canada where it is being used to reduce doxorubicin-induced cardiotoxicity.[1,2] In the USA, due to concerns that dexrazoxane might reduce the antitumor efficacy of doxorubicin,[3] it is labeled for use with women who have already received a cumulative doxorubicin dose of 300 mg/m^2. Under physiological conditions, dexrazoxane undergoes ring-opening hydrolysis to ADR-925,[4,5] an analog of EDTA. Dexrazoxane likely exerts its cardioprotective effects through its rings-opened hydrolysis product by virtue of its ability to strongly chelate free iron,[6] or to quickly and efficiently remove iron from its complex with doxorubicin,[7] thus reducing doxorubicin-induced iron-based oxygen free radical damage.[8]

Dexrazoxane is the (+)-(*S*)-enantiomer of racemic ICRF-159, which was originally developed as an antitumor agent.[9,10] It has recently been found that the bisdioxopiperazines [including dexrazoxane, ICRF-159 (razoxane), ICRF-154 and ICRF-193] are strong inhibitors of mammalian DNA topoisomerase II.[11–13] 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] Doxorubicin[15] and daunorubicin,[16] like etoposide and amsacrine, are thought to inhibit cell growth by virtue of their ability to stabilize a covalent topoisomerase II–DNA intermediate (the cleavable complex).[14,17,18]

Supported in part by the Medical Research Council of Canada and the Natural Sciences and Engineering Research Council of Canada.

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However, the bisdioxopiperazines,[11,12] like several other drugs that are known to act on topoisomerase II, including suramin,[19] merbarone[20] and aclarubicin,[21] have been shown to inhibit topoisomerase II *in vitro*[11,22] and in cultured cells,[12] without inducing cleavable complex formation. The bisdioxopiperazines can, in fact, reduce protein–DNA cross-links induced by etoposide, amsacrine, daunorubicin and doxorubicin.[11,12,22] They may do this by trapping the enzyme in the form of a closed protein clamp,[23] thus preventing the formation or stabilization of the topoisomerase II–DNA intermediate.

A variety of studies[22,24–28] have produced conflicting results on whether dexrazoxane (and other bisdioxopiperazines) add to or antagonize the *in vitro* growth inhibitory effects of doxorubicin and daunorubicin. Because both dexrazoxane and doxorubicin act on topoisomerase II, this study was undertaken to investigate under what conditions dexrazoxane could add to or antagonize doxorubicin- and daunorubicin-mediated growth inhibition.

Materials and methods

Cell culture and growth inhibition assays

Chinese hamster ovary (CHO) cells (type AA8; ATCC CRL-1859), obtained from the American Type Culture Collection (Rockville, MD), were grown in α-minimum essential medium (α-MEM; Gibco/BRL, Burlington, Canada) containing 20 mM HEPES (Sigma, St Louis, MO), 100 units/ml penicillin G, 100 μg/ml streptomycin, 10% calf serum (Gibco, iron supplemented and enriched) in an atmosphere of 5% CO_2 and 95% air at 37°C (pH 7.4).

Cells in exponential growth were harvested and seeded (typically 2000 cells/well) in 96-well microtiter plates (100 μl/well) and allowed to attach for 24 h. The drugs were dissolved either in α-MEM (dexrazoxane) or DMSO (ICRF-193), and were added to give a final volume of 200 μl/well. When DMSO was used, the final concentration of DMSO did not exceed 0.5% (v/v). This amount of DMSO was shown through the use of appropriate controls to have no significant effect on cell growth. In experiments in which the cells were washed to remove drugs, the drugged medium was carefully aspirated off and 100 μl of fresh undrugged medium was added. After 15 min this was repeated, and after another 15 min, 200 μl of fresh medium was added and the cells were then allowed to grow for the times indicated. The cell growth was determined by MTT assay.[29] Briefly, 20 μl of MTT (2.5 mg/ml in PBS, Dulbecco's phosphate buffered saline) was added to each well and the plate was incubated for a further 4 h. After careful aspiration of the medium, 100 μl of DMSO was added and the absorbance was read at 490 nm in a Molecular Devices (Menlo Park, CA) plate reader with reference to the absorbance at 650 nm and appropriate blanks. 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-parameter logistic equation with the limiting MTT absorbance at high drug concentration set to zero.

In the clonogenic assay the cells (typically 2.5×10^6 cells) in log phase growth were seeded in 25 cm^2 T-flasks and allowed to attach for 24 h. After the medium was replaced, the cells were preincubated with dexrazoxane (or not) for 20 min at 37°C in the incubator. The anthracyclines were then added (or not) and the incubation was continued for 1 h. After rinsing twice with PBS to remove the drugs, the cells were trypsinized and plated in 100 mm culture plates. After 10–12 days the colonies (larger than 50 cells) were stained with Wright–Giemsa stain and counted. The cloning efficiency was typically 40%. The percentage cell survival was calculated as a percentage of the control experiment (no added drugs) that was run at the same time.

Drugs

Dexrazoxane and doxorubicin were gifts from Pharmacia & Upjohn (Columbus, OH). Daunorubicin was a gift from Rhône-Poulenc Pharma (Montreal, Canada). ICRF-193 was prepared essentially as described in the published literature[30] except that dimethylglyoxime was used as a starting material.

Results

Antagonism of anthracycline growth inhibition by dexrazoxane using a clonogenic assay

As shown in Figure 2, the growth inhibitory effects of both doxorubicin and daunorubicin were strongly antagonized when dexrazoxane was preincubated with the attached cells for 20 min before the anthracycline was added. The cells were incubated with both drugs for a further 1 h and the

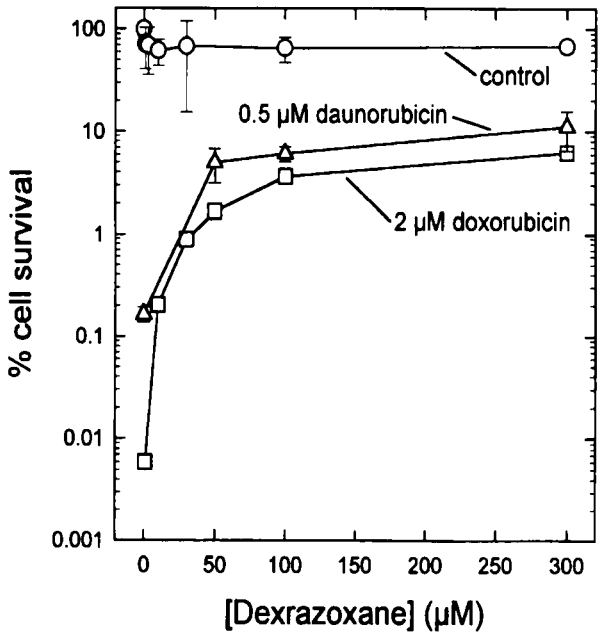

Figure 2. Antagonism of doxorubicin and daunorubicin growth inhibition by various concentrations of dexrazoxane using a clonogenic assay. The attached cells were preincubated with dexrazoxane for 20 min at 37°C at the concentrations indicated and then treated with for 1 h with either 2.0 μM doxorubicin (□), 0.5 μM daunorubicin (△); or not (○). The error bars are SDs from three replicate determinations. While the results shown are from a single experiment, the results are typical of three experiments. Where the error bars are not displayed, they are smaller than the size of the symbol.

drugs were removed by washing the attached cells twice. Preincubation of the cells with 300 μM dexrazoxane increased the cell survival 1000-fold for doxorubicin-treated (2 μM) cells and 70-fold for daunorubicin-treated (0·5 μM) cells. Preincubation with dexrazoxane did not, however, result in 100% antagonism of either doxorubicin- or daunorubicin-mediated growth inhibition at even the highest concentration of dexrazoxane used. Brief exposure (1.3 h) of the cells to even the highest concentration of dexrazoxane (300 μM) did not have much effect on cell survival (approximately 70% survival). The influence of dexrazoxane on the growth inhibition effects of doxorubicin and daunorubicin were investigated in further detail using a MTT microtiter plate assay to determine cell growth.

The effect of continuous exposure of dexrazoxane and anthracycline on CHO cell growth

In the experiments shown in Figure 3(a and b), dexrazoxane (0–3 μM) was preincubated with the

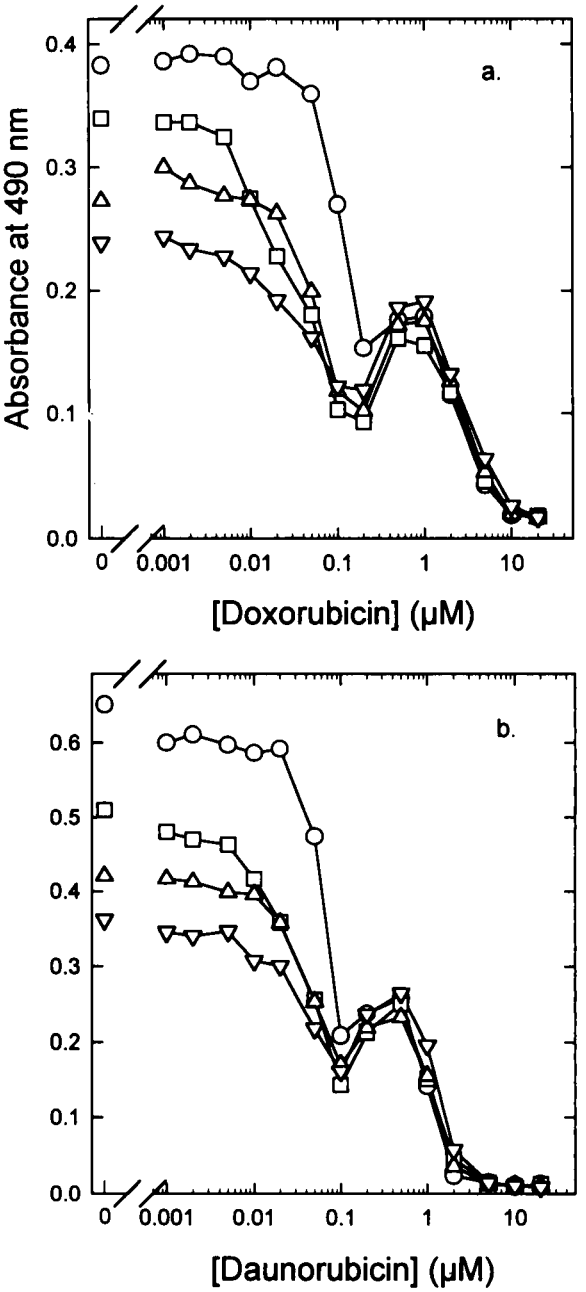

Figure 3. The effect of dexrazoxane on anthracycline growth inhibition with continuous exposure to both drugs. Dexrazoxane was preincubated with the cells for 20 min before the addition of anthracycline. The CHO cells were continuously incubated with both drugs for 48 h and then assayed with MTT. (a) Inhibition of growth of CHO cells by doxorubicin in the absence (○) and presence of 1.5 μM (□), 2.0 μM (△) or 3.0 μM (▽) dexrazoxane. (b) Inhibition of growth of CHO cells by daunorubicin in the absence (○) and presence of 1.5 μM (□), 2.0 μM (△) or 3.0 μM (▽) dexrazoxane. The absorbance values to the left of the axis break on a linear scale are controls that were obtained in the absence of anthracycline. The data points are averages of replicates in six wells. The error bars have been omitted for clarity.

attached cells for 20 min before the anthracycline was added. The cells were allowed to grow in the presence of both drugs for a further 48 h before cell survival was determined using an MTT assay. This range of dexrazoxane concentrations was chosen because the IC_{50} for continuous exposure of dexrazoxane to CHO cells is 3 μM.[13] Below 0.1 μM anthracycline, there was an additive effect on the growth inhibition of both anthracyclines (Figure 3). Above approximately 0.1 μM anthracycline, dexrazoxane did not significantly affect anthracycline growth inhibition. It was observed to be a highly reproducible feature of the anthracycline cell survival curves that partial stimulation of growth was observed in the low micromolar range. This effect has been noted previously.[31,32]

Effect of preincubation and brief exposure of the cells to dexrazoxane and anthracycline on growth inhibition

In the experiments shown in Figure 4(a and b), various concentrations of dexrazoxane were added to the cells 20 min prior to the addition of the anthracycline. The attached cells were then incubated with both drugs for 1 h. The cells were then washed twice over a 30 min period with drug-free medium and then grown for 48 h. In contrast to the data shown in Figure 3(a and b), these results show that a brief preincubation of the cells with higher concentrations of dexrazoxane results in antagonism of the growth inhibitory effects of the anthracyclines over much of the survival curve. These results are consistent with the antagonism shown in the clonogenic experiments of Figure 2. The IC_{50}'s (Table 1), obtained from the data in Figure 4, show that the antagonism is only seen at dexrazoxane concentrations 50 μM and above. At 100 μM dexrazoxane the IC_{50} increased 3.4-fold for doxorubicin and 3.8-fold for daunorubicin (Table 1). As shown by the controls in which anthracycline was absent (Figure 4a and b), dexrazoxane was quickly and effectively removed by washing and thus did not contribute to the overall cytotoxicity. Dexrazoxane would be highly growth inhibitory (IC_{50} 3 μM) if significant amounts of dexrazoxane had remained either in the cells or the medium.[13]

Effect of the concentration of dexrazoxane and ICRF-193 on doxorubicin and daunorubicin growth inhibition

Growth inhibition experiments were also conducted in which the anthracycline concentration was fixed (to give approximately 10–20% cell survival)

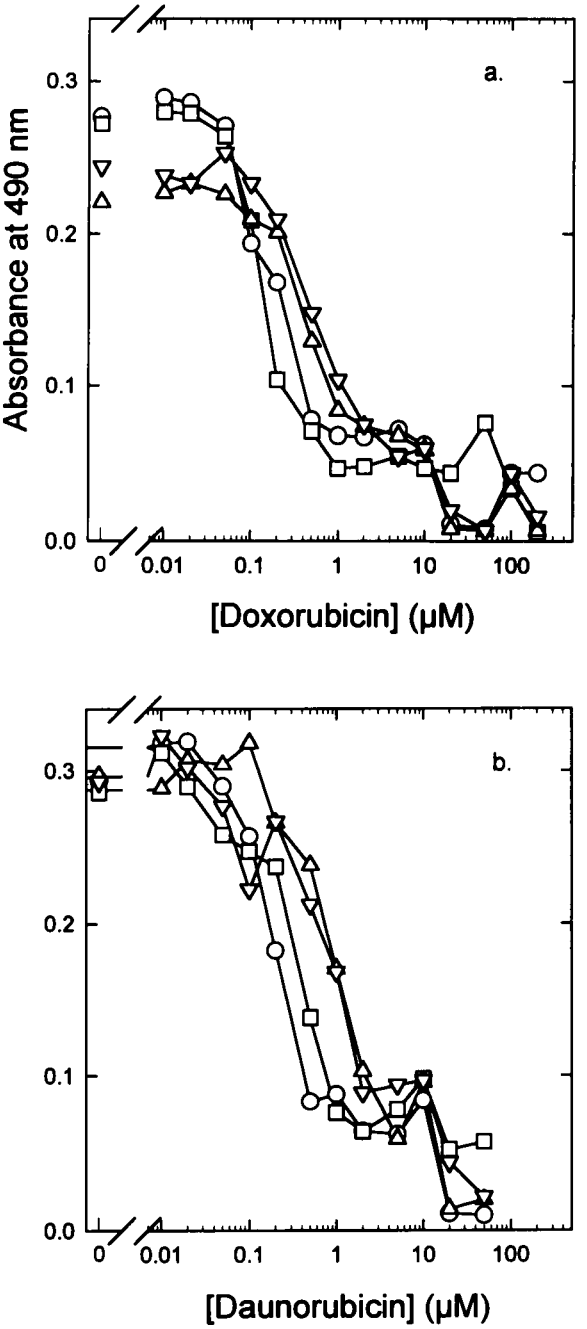

Figure 4. Anthracycline growth inhibition following preincubation with dexrazoxane. Inhibition of growth of CHO cells by (a) doxorubicin and by (b) daunorubicin. These experiments were conducted in the absence (○) and presence of 10 μM (□), 50 μM (△) or 100 μM (▼) dexrazoxane. Dexrazoxane was added 20 min prior to the addition of doxorubicin. The anthracyclines were then added and the cells were incubated for 1 h. The cells were then washed two times with fresh medium over 30 min, allowed to grow for 48 h and assayed with MTT. The absorbance values at the left of the axis break are controls that were obtained in the absence of any anthracycline. The data points are averages of replicates in six wells. The error bars are omitted for clarity.

Table 1. Effect of a brief preincubation and brief exposure to dexrazoxane on anthracycline-mediated growth inhibition of CHO cells

Dexrazoxane (μM)	IC_{50} for doxorubicin[a] (μM)	IC_{50} for daunorubicin[a] (μM)
0	0.27 ± 0.12	0.33 ± 0.10
10	0.20 ± 0.08	0.55 ± 0.24
50	0.85 ± 0.24	1.50 ± 0.30
100	0.91 ± 0.23	1.24 ± 0.36

[a]Concentration of the anthracycline that caused a 50% reduction in growth inhibition of CHO cells as measured by MTT assay. In these experiments dexrazoxane was added to the attached cells 20 min before the anthracycline was added. Both drugs were incubated together for a further 1 h. The cells were then washed two times over 30 min and allowed to grow for a further 48 h. Errors are ± SEM from a three-parameter logistic fit.

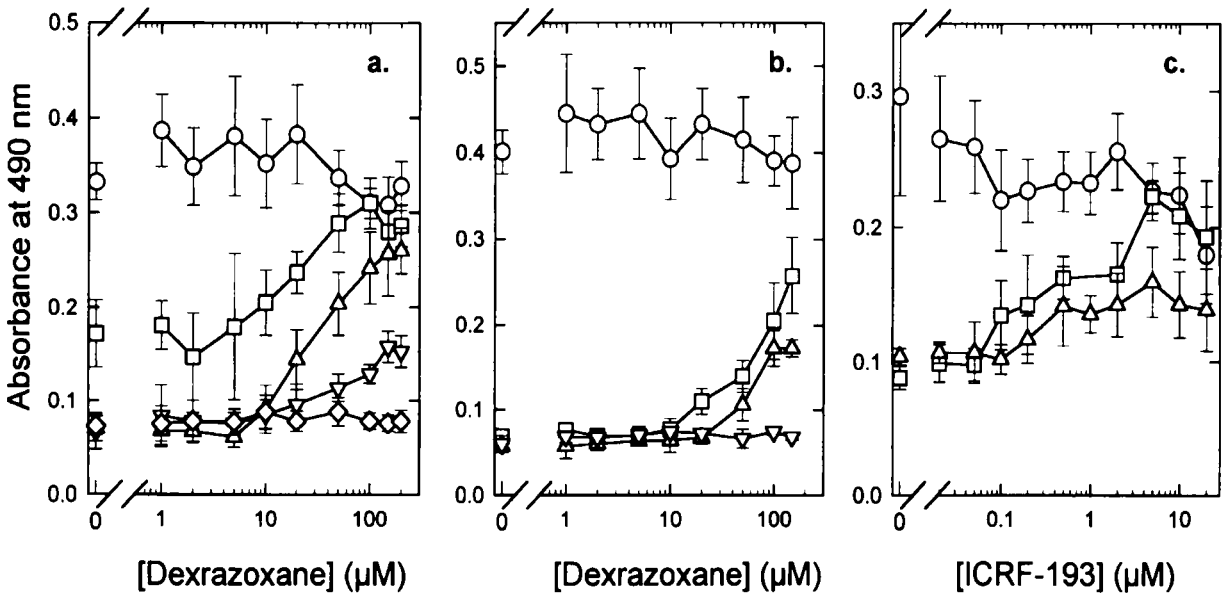

Figure 5. Effect of dexrazoxane and ICRF-193 concentration on doxorubicin and daunorubicin growth inhibition after a 20 min preincubation of the bisdioxopiperazine. (a) Absorbance at 490 nm as a function of dexrazoxane concentration in the absence (○) and presence of 0.2 μM (□), 0.5 μM (△), 1.0 μM (▽) and 5.0 μM (◇) doxorubicin. (b) Absorbance at 490 nm as a function of dexrazoxane concentration in the absence (○) and presence of 1 μM (□), 2.0 μM (△) or 2.0 μM (▽) daunorubicin. In the experiment denoted by the symbol (▽), the cells were washed twice before daunorubicin was added. (c) Absorbance at 490 nm as a function of ICRF-193 concentration in the absence (○); and presence of 1.0 μM (□) or 2.0 μM (▽) daunorubicin. The data points plotted to the left of the axis break on a linear scale are controls in the absence of any added dexrazoxane. The error bars are SDs. The data points are averages (± SEM) of replicates in six wells.

and the dexrazoxane or ICRF-193 concentration was varied. This was done in order to determine the concentration at which bisdioxopiperazine antagonism occurs and to elucidate the mechanism of the antagonism. Dexrazoxane and ICRF-193 have been shown to inhibit topoisomerase II with IC_{50}'s of 13 and 0.6 μM, respectively.[13] Thus, if antagonism is due to bisdioxopiperazine inhibition of topoisomerase II, ICRF-193 should be antagonistic at a correspondingly lower concentration than dexrazoxane. In these experiments the bisdioxopiperazine was incubated with the cells 20 min prior to the addition of anthracycline. The 1 h incubation with anthracycline was then followed by two washes with medium. Figure 5(a and b) shows that at dexrazoxane concentrations in the 20–200 μM range strong, though not complete, antagonism, of both doxorubicin and daunorubicin growth inhibition is seen. It can also been seen from the data of Figure 5(a and b) that at higher anthracycline concentrations (above 0.5 μM for doxorubicin, above 2 μM for daunorubicin) dexra-

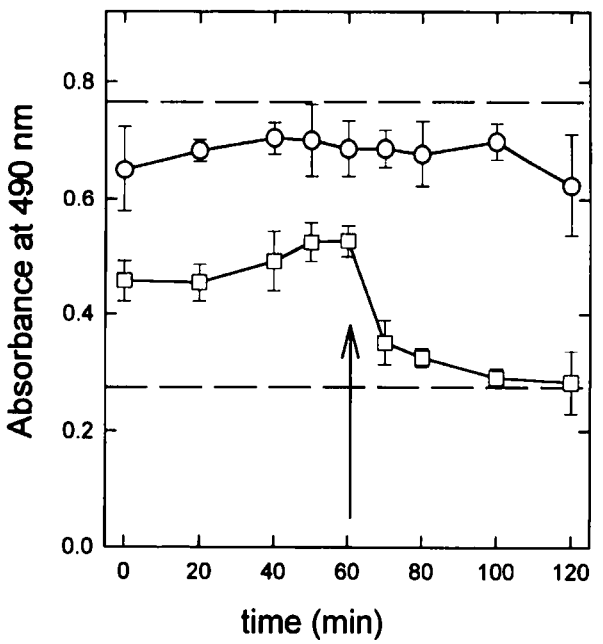

Figure 6. Effect of time of preincubation or postincubation of 100 μM dexrazoxane on the growth of cells in the presence of 1 μM daunorubicin (□) or not (○). For *t* less than 60 min dexrazoxane was added before daunorubicin and for *t* greater than 60 min dexrazoxane was added after daunorubicin at the times indicated. In all cases the cells were incubated with daunorubicin for 1 h. At the time marked by the arrow, all wells were drugged with daunorubicin for a constant time of 1 h. The cells were then washed two times with medium before being allowed to grow for a further 48 h. The upper broken line is the control absorbance in the absence of either drug and the lower broken line is the absorbance in the absence of dexrazoxane. The cells were initially seeded at 5000 cells/well, 24 h prior to drugging. Antagonism is seen when the cells are preincubated with dexrazoxane (times from 0 to 60 min) and little to no antagonism when dexrazoxane is added after daunorubicin is added (times greater than 60 min). The data points are averages (± SEM) of replicates in six wells.

zoxane was unable to antagonize anthracycline growth inhibition. Also shown in Figure 5(b) are the results of an experiment (denoted by inverted triangles) in which the cells were washed two times just after the 20 min dexrazoxane incubation and just prior to the addition of daunorubicin. These results showed that when dexrazoxane was washed out of the cells before daunorubicin was added, dexrazoxane did not antagonize daunorubicin growth inhibition. This result indicates that dexrazoxane must be present in the cells to antagonize daunorubicin growth inhibition and that the addition of dexrazoxane to the cells did not induce irreversible cellular processes that result in antagonism. ICRF-193 strongly antagonized dauno-rubicin growth inhibition in the 0.2–10 μM concentration (Figure 5c). ICRF-193 could not, however, be shown to antagonize doxorubicin growth inhibition to any significant degree (data not shown) before cytotoxic effects of ICRF-193 were noted. The more lipophilic ICRF-193 may be less easily washed out of the cells than dexrazoxane, resulting in some ICRF-193-mediated growth inhibition at higher ICRF-193 concentrations.

Effect of incubating dexrazoxane with cells for various times either before or after daunorubicin is added

In order to more clearly define the time period over which dexrazoxane exerts its antagonism, experiments were designed in which 100 μM dexrazoxane was added to attached cells at various times up to 1 h before and 1 h after daunorubicin was added. In these experiments, the daunorubicin incubation time was maintained constant at 1 h. After the drug incubations, the cells were washed two times and grown for another 48 h. As the data in Figure 6 shows, the addition of dexrazoxane even 0.5 min (denoted by the vertical arrow) before daunorubicin was added resulted in as much antagonism as when it was added 1 h before daunorubicin was added. This indicated that the entry of dexrazoxane into the cell and its binding to its target was rapid. This data also showed that the addition of dexrazoxane, even 10 min or longer after the addition of daunorubicin, resulted in the loss of most of the antagonism. These results indicated that the committed cellular processes that daunorubicin causes that resulted in growth inhibition were initiated within just a very few minutes of entry of daunorubicin into the cell. Dexrazoxane is unable to reverse these daunorubicin-mediated processes once they have been initiated.

Discussion

Dexrazoxane and ICRF-193 have both been shown to inhibit topoisomerase II.[11–13] A quantitative structure–activity relationship study[13] using 12 structurally related bisdioxopiperazines demonstrated a strong correlation of CHO growth inhibi-

tion with strength of topoisomerase II inhibition, indicating that bisdioxopiperazine-mediated cell growth inhibition occurs through the inhibition of topoisomerase II. It was shown on studies with yeast topoisomerase II that the dexrazoxane analog ICRF-193, in an ATP-dependent reaction, traps the enzyme in its closed clamp form, preventing its binding to DNA.[23] This study showed that when a low concentration of dexrazoxane was added to the cells before the addition of doxorubicin or daunorubicin and both drugs were continuously incubated with the cells, the growth inhibitory effects of the two drugs was additive (Figure 3a and b) at anthracycline concentrations below about 0.1 μM, and had no effect above 0.1 μM. This effect may be due to the ability of both doxorubicin and daunorubicin to abolish topoisomerase II-mediated DNA cleavage above about 0.5 μM anthracycline concentration.[15] Because the growth inhibition of dexrazoxane depends upon it being present for a significant fraction of the cell cycle,[33] a brief exposure to dexrazoxane causes little growth inhibition. Thus, in experiments using both a clonogenic and MTT assay, preincubation of the cells with dexrazoxane for a short period of time resulted in significant antagonism of both doxorubicin and daunorubicin growth inhibition. These results are consistent with dexrazoxane quickly entering the cell and locking the enzyme into its closed clamp DNA-unreactive form, thereby preventing daunorubicin from stabilizing the covalent topoisomerase II–DNA intermediate.[23]

The results of this study also indicated that when the anthracycline was allowed to enter the cell first, the anthracycline quickly initiated a series of biochemical processes that resulted in growth inhibition. This likely involved the stabilized covalent topoisomerase II–DNA intermediate that results in the formation of a cellular toxin.[14] The results shown in Figure 6 indicated that these processes cannot be reversed by the subsequent addition of dexrazoxane; they can only be prevented from occurring if sufficiently high concentrations of dexrazoxane are present before the anthracycline is added.

The lack of bisdioxopiperazine growth inhibition at even high bisdioxopiperazine concentrations for short (up to 2 h) exposure times (Figure 5) allowed us to determine the concentration dependence of dexrazoxane and ICRF-193-mediated antagonism of daunorubicin growth inhibition. The results shown in Figure 5 indicated that this antagonism was produced in the 20–200 μM concentration range for dexrazoxane and the 0.1–20 μM concentration range for ICRF-193. The lower values compare well to the IC_{50}'s for dexrazoxane and ICRF-193 topoisomerase II inhibition of 13 and 0.6 μM, respectively.[13] This agreement is good evidence that the bisdioxopiperazines antagonized anthracycline growth inhibition through their inhibition of topoisomerase II. This same study[13] also measured the IC_{50}'s for CHO cell growth inhibition to be 3 and 0.017 μM for dexrazoxane and ICRF-193, respectively.

It has been shown[34] that ICRF-159 (the racemate of dexrazoxane) passively diffuses in and is efficiently washed out of BHK-21S cells with a half-time of 20 min. Our results showed that with two washes over 30 min, the dexrazoxane concentration was brought down from 200 μM to below growth inhibitory concentrations (below 2 μM). Also when cells that had been preincubated with dexrazoxane were washed just before daunorubicin was added (Figure 5b), no antagonism of daunorubicin growth inhibition was seen. Together these results show that the inhibitory effect of dexrazoxane on topoisomerase II was reversible. Neither doxorubicin nor daunorubicin are efficiently washed out of cells,[27,35] probably due to the high affinity of the anthracyclines for DNA and other cell components.

Several other studies have investigated the effect of dexrazoxane and other bisdioxopiperazines on anthracycline growth inhibition. In agreement with this study it was shown[22] using a clonogenic assay, that when dexrazoxane is added 5 min prior to the addition of daunorubicin, significant antagonism of daunorubicin growth inhibition is seen. Antagonism could not, however, be demonstrated for doxorubicin, though dexrazoxane-mediated inhibition of both doxorubicin- and daunorubicin-induced DNA single-strand breaks and doxorubicin-induced DNA–protein cross-links were seen. Dexrazoxane was not able to antagonize the growth inhibitory effects of higher concentrations of either doxorubicin or daunorubicin (Figures 4a, 4c, 5a and 5b). This may be due to doxorubicin also being able to inhibit cell growth through other non-topoisomerase II-mediated mechanisms.[36] In other studies[26,27] it has been reported that a 1 h incubation with dexrazoxane and doxorubicin produces additive growth inhibition, while a 24 h incubation gave synergistic growth inhibition. However, because the order in which the drugs were added was not specified, a direct comparison with this study is not possible. Both ICRF-159 and ICRF-186 [the (−)-*R*-isomer of dexrazoxane] were shown[24] to antagonize the growth inhibition of

daunorubicin, but not doxorubicin, using a 24 h drug exposure. Because the order of addition was not specified, a direct comparison is again not possible. Dexrazoxane has also been shown to synergistically potentiate doxorubicin growth inhibition in HL-60 cells,[37] whether dexrazoxane was preincubated or not prior to the addition of doxorubicin. More recently[28] dexrazoxane was found not to have any synergistic activity in combination with doxorubicin in a 3 day continuous exposure to drugs. In these experiments as well, the order of addition of the drugs was not specified. The bisdioxopiperazine ICRF-154 was shown[25] to have supra-additive effects in combination with doxorubicin in a 3 day incubation. ICRF-193 has also been shown to antagonize mitoxantrone and teniposide-induced cellular apoptosis if added simultaneously; but no rescue was seen if it was added 2 h later.[38] Conflicting results presented in these previous publications may be partly explained by the factors that we have identified here that determine whether dexrazoxane is antagonistic or synergistic to anthracycline growth inhibition.

Whether or not dexrazoxane can antagonize doxorubicin growth inhibition *in vivo* might depend on the relative pharmacokinetics and tumor uptake by both of these drugs. Typically, dexrazoxane is clinically given at 600 mg/m^2 30 min prior to doxorubicin dosing (60 mg/m^2).[39] Dexrazoxane has an elimination phase $t_{1/2}$ of 4.2 ± 2.9 h and yields a peak plasma concentration of 340 ± 80 μM.[35] This concentration is about 20 times larger than the minimum concentration of dexrazoxane required to significantly antagonize doxorubicin-mediated growth inhibition (Figure 5a). Thus, if this plasma concentration of dexrazoxane is even partially obtained in the tumor, dexrazoxane may bind to topoisomerase II and antagonize the ability of doxorubicin to interact with topoisomerase II. In addition, the short $t_{1/2}$ for dexrazoxane would reduce the antitumor effectiveness of dexrazoxane, which must be present over a significant fraction of the cell cycle to exert its growth inhibitory effects. Clinical trials with women with advanced breast cancer[1] showed that when dexrazoxane was used to protect against doxorubicin-induced cardiotoxicity, the antitumor activity of doxorubicin is not decreased by the addition of dexrazoxane to the protocol. However, the increased doses of doxorubicin that could be used could not be shown to result in significant increases in time to tumor progression or overall survival. In an unpublished trial with women with advanced breast cancer, the addition of dexrazoxane to the protocol resulted in a statistically significant lower response rate (48 versus 63%).[3] However, it should also be noted that using a wide variety of implanted tumors in an animal model, dexrazoxane has been shown[40] not to interfere with the antitumor activity of doxorubicin. It has also been shown[41] that in an implanted tumor mouse model, the combination of ICRF-159 and either doxorubicin or daunorubicin resulted in significant increases in life span when ICRF-159 was administered (i.p.) 5 min before the anthracycline. The results of this study suggest that if the dexrazoxane dose were given after the administration of the anthracycline, the dexrazoxane-modulated antagonism might be reduced. However, it is unclear whether any therapeutic advantage could result from this dosing protocol, as it has been shown[42] in a dog model, that even giving dexrazoxane 2 h after doxorubicin results in significantly less cardioprotection than when the two drugs are given simultaneously.

Summary

This study has shown that whether or not dexrazoxane displays antagonism towards anthracycline-mediated growth inhibition critically depends on a number of factors. The order of addition of the drugs was found to be critically important, with antagonism seen only when dexrazoxane was added to the cells first. However, dexrazoxane, at lower concentrations, exhibited additive growth inhibition with the anthracyclines when the cells were continuously incubated with both drugs. The concentration of dexrazoxane was also important, with concentrations of 50 μM or more being required for antagonism of anthracycline growth inhibition. The concentration of anthracycline was also important as dexrazoxane was not able to antagonize anthracycline growth inhibition at higher concentrations of anthracyclines (above 0.5 μM for doxorubicin; above 2 μM for daunorubicin). It was also shown that ICRF-193, which is a much stronger topoisomerase II inhibitor than ICRF-187, antagonized daunorubicin growth inhibition at correspondingly lower concentrations than did ICRF-187. The addition of dexrazoxane to the cells at various times before and after daunorubicin was added showed that the anthracycline-mediated cellular processes that are initiated that result in growth inhibition occur over a period of only a few minutes. Further, it was shown that the anthracycline-induced growth inhi-

bition cannot be reversed by the subsequent addition of dexrazoxane. These results are consistent with dexrazoxane locking topoisomerase II into its closed-clamp form,[23] preventing its binding to DNA and the formation of the anthracycline-induced stabilized topoisomerase II–DNA complex.

Acknowledgments

The skilled technical assistance of Mukhtiar Singh and Steven Smith is gratefully acknowledged.

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(Received 19 March 1996; accepted 30 April 1996)