Dexrazoxane Protects against Myelosuppression from the DNA Cleavage–Enhancing Drugs Etoposide and Daunorubicin but not Doxorubicin

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Abstract

Purpose: The anthracyclines daunorubicin and doxorubicin and the epipodophyllotoxin etoposide are potent DNA cleavage–enhancing drugs that are widely used in clinical oncology; however, myelosuppression and cardiac toxicity limit their use. Dexrazoxane (ICRF-187) is recommended for protection against anthracycline-induced cardiotoxicity.

Experimental Design: Because of their widespread use, the hematologic toxicity following coadministration of dexrazoxane and these three structurally different DNA cleavage enhancers was investigated: Sensitivity of human and murine blood progenitor cells to etoposide, daunorubicin, and doxorubicin ± dexrazoxane was determined in granulocyte-macrophage colony forming assays. Likewise, in vivo, B6D2F1 mice were treated with etoposide, daunorubicin, and doxorubicin, with or without dexrazoxane over a wide range of doses: posttreatment, a full hematologic evaluation was done.

Results: Nontoxic doses of dexrazoxane reduced myelosuppression and weight loss from daunorubicin and etoposide in mice and antagonized their antiproliferative effects in the colony assay; however, dexrazoxane neither reduced myelosuppression, weight loss, nor the in vitro cytotoxicity from doxorubicin.

Conclusion: Although our findings support the observation that dexrazoxane reduces neither hematologic activity nor antitumor activity from doxorubicin clinically, the potent antagonism of daunorubicin activity raises concern; a possible interference with anticancer efficacy certainly would call for renewed attention. Our data also suggest that significant etoposide dose escalation is perhaps possible by the use of dexrazoxane. Clinical trials in patients with brain metastases combining dexrazoxane and high doses of etoposide is ongoing with the aim of improving efficacy without aggravating hematologic toxicity. If successful, this represents an exciting mechanism for pharmacologic regulation of side effects from cytotoxic chemotherapy.

Topoisomerase II transiently cleaves double-stranded DNA to allow the passage of another unbroken DNA strand after which the cleaved strands are religated. Drugs that inhibit the enzyme can be broadly categorized into catalytic inhibitors and DNA cleavage–enhancing drugs, depending on their site of action in the catalytic cycle of the enzyme (1). The DNA cleavage–enhancing drugs etoposide, daunorubicin, and doxorubicin stabilize the DNA-enzyme complex in its cleaved conformation and inhibit resealing (2–4), leading to DNA double-strand breaks and cell death (5). It is believed that these double-strand breaks are responsible for cell death. The bisdioxopiperazine dexrazoxane is used to ameliorate anthracycline-induced damage to the heart (6, 7) and is believed to do so because of the iron-chelating properties of its ring-opened metabolite ADR-925 (8, 9). The parent compound, the ring-closed form, is, however, also a potent topoisomerase II catalytic inhibitor (10–12); in contrast to the DNA cleavage–enhancing drugs, it stabilizes the DNA-enzyme complex in a “closed clamp” conformation, rendering the enzyme less sensitive to the cleavage enhancers (11). Thus, it inhibits the enzymatic activity without the induction of DNA strand breaks. A plenitude of potential applications of dexrazoxane has been investigated (8) and the effect of combination treatment with the poisons continues to be an area of active research. In preclinical models, dexrazoxane, or other bisdioxopiperazines, protected against chemotherapy-induced toxicity; in mice with brain tumors, dexrazoxane reduced toxicity from high doses of etoposide and increased the treatment efficacy (13). Hematologic toxicity from the epipodophyllotoxins and the anthracyclines is frequent and sometimes fatal. Gastrointestinal toxicity and mucositis are also common side effects (14), besides of course cardiotoxicity from the anthracyclines, which limits their total dose (15). Considering the widespread
use clinically of these drugs, we decided to study the effect of coadministration of dexrazoxane in combination with different structural classes of commonly used DNA cleavage-enhancing drugs (i.e., etoposide, daunorubicin, and doxorubicin): In vivo myelosuppression in terms of relevant parameters [i.e., WBC, absolute neutrophile count (ANC), platelets, and RBC] was recorded, as well as general toxicity expressed as changes in bodyweight. In vitro, the effects of coinoculation was investigated in granulocyte-macrophage colony-forming unit (GM-CFU) assays using both human and murine bone marrow cells.

Materials and Methods

Materials. Dexrazoxane was from either Chiron Corporation (Amsterdam, the Netherlands) or Pfizer (Kalamazoo, MI). Etoposide and doxorubicin were from Pfizer A/S (Copenhagen, Denmark) and daunorubicin was from Rhone-Poulenc Rohrer (Holte, Denmark). Fentanyl-fluanisone (hypnorm) was from Janssen-Cilag (Bierkeroed, Denmark) and midazolam (dormicum) was from Roche (Hvidovre, Denmark). All drugs were commercially obtained.

Cell culture. Methocult contained 1% methylcellulose in Iscove’s modified Dulbecco’s medium, 1% bovine serum albumin, 100 nmol/L 2-mercaptoethanol, 2 mmol/L l-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. To support human cells, 30% fetal bovine serum, 50 ng/mL rh Stem Cell Factor, 10 ng/mL rh IL-3, and 10 ng/mL rh GM-CSF were added; to support murine cells, 15% fetal bovine serum, 10 μg/mL rh insulin, 200 μg/mL human transferrin (iron not saturated), 50 ng/mL rm Stem Cell Factor, 10 ng/mL rm IL-3, and 10 ng/mL rh IL-6 were added. Iscove’s modified Dulbecco’s medium and antibiotics were from Life Technologies (Taastrup, Denmark). Methocult and other reagents were obtained from StemCell Technologies, Inc. (Vancouver, BC, Canada).

In vivo procedures. B6D2F1 female mice, weighing 19 to 22 grams, were obtained from Taconic M&B (Ry, Denmark). They were kept in a light- and temperature-controlled environment with ad libitum access to water and standard laboratory diet. Experiments were conducted according to institutional and national guidelines for the care and use of laboratory animals, and approved by the Danish Experimental Animal Inspectorate, Department of Justice.

In vitro granulocyte-macrophage colony-forming unit assay. Mice (B6D2F1) were sacrificed with CO2 and bone marrow cells obtained by flushing the femoral bone cavity with Iscove’s modified Dulbecco’s medium with 10% fetal bovine serum at 37°C. Cells were then cooled at 1°C per minute until −180°C in a freezing solution containing 7.5% DMSO. Human CD34+ bone marrow cells from healthy American volunteers were obtained from BioWhittaker.

Fig. 1. Dose-response curves in the GM-CFU assay obtained after incubation in increasing concentrations of etoposide, daunorubicin, doxorubicin, or dexrazoxane alone, using human (△) and murine (●) bone marrow cells. The numbers of colonies are percentage of controls and represent the means of two or more experiments with duplicate plating. Bars, SE. A, etoposide; B, daunorubicin; C, doxorubicin; D, dexrazoxane.
Molecular Applications ApS (Vallensbaek, Denmark). All cells were kept at 18°C and prepared identically for use; after washing twice in 37°C medium, they were left for 1 hour to rest at 37°C and then stained with 0.1% nigrosin. The fractions of dead human and murine cells were 6% and 9.4%, respectively (not statistically different). Incubation in dexrazoxane alone was 80 minutes, and in etoposide, doxorubicin, or daunorubicin alone 60 minutes. In two-drug experiments, cells were incubated in dexrazoxane alone for 20 minutes, etoposide, doxorubicin, or daunorubicin was then added and cells were coincubated for further 60 minutes. Cells were then washed twice in Iscove’s modified Dulbecco’s medium with 10% FCS, diluted in Iscove’s modified Dulbecco’s medium with 2% FCS, and mixed with Methocult 1:10 before plating. Murine (2.5 × 10⁵) or human cells (1 × 10⁶) were plated in duplicate. Murine and human colonies (>50 cells) were counted day 12 and 16, respectively, and the mean colony counts in controls were 91 (SD 16) and 153 (SD 25).

**Determination of myelosuppression in vivo.** Etoposide and dexrazoxane were both administered by the i.p. route. Doxorubicin and daunorubicin were given by slow i.v. infusion over 1 minute in a tail vein. Dexrazoxane, or saline control, was administered 20 minutes before cytotoxics. A final volume to be administered of 0.2 mL for all drugs was obtained by diluting the drugs accordingly. The drug doses tested were chosen based on their observed in vitro activity. Conversion of murine doses in mg/kg to human doses in mg/m² by adjusting for the surface area to weight ratio (mg/m² = km × mg/kg; with the conversion factor km for mice being set to 3) revealed that, e.g., a dose of dexrazoxane in mice of 125 mg/kg corresponded to a clinically relevant dose in humans of 375 mg/m². The ratios of the drugs used in combination, e.g., 125 mg/kg dexrazoxane combined with 10, 15, or 20 mg/kg doxorubicin resulted in dexrazoxane-to-doxorubicin ratios of 6.25, 8.3, and 12.5, respectively, and these are close to the clinically recommended ratio of 10:1.

Before sampling, mice were anesthetized i.p. using 0.05 mL/10 g of a solution of 1 part fentanyl-fluanisone, 1 part midazolam, and 2 parts isotonic saline, and were placed under a heating lamp to antagonize cooling and peripheral vasoconstriction and to facilitate bleeding. The tail-tip was cut off with surgical scissors and 250 µL blood was collected in a K2-EDTA–coated microtainer from Becton-Dickinson (Broendby, Denmark). Bleeding was controlled by compression. Hematologic analysis was done (Scantox, Ejby, Denmark) on an ABX Pentra 120 automated haematology analyzer (ABX Diagnostics, Montpellier, France). For the differential count, a manual count of a minimum of 100 cells on each smear was done.

**Statistical analysis.** The blood cell counts were analyzed by one-way ANOVA. P < 5% were considered significant. Pair-wise t tests were done only if the ANOVA model was significant (P < 0.05) and corrected for multiple testing using the Bonferroni adjustment. The levels of statistical significance were defined as follows: ★ P < 0.05; ★★ P < 0.01; and ★★★ P < 0.001; ns., not statistically significant (P ≥ 0.05). The statistical software package SAS 8.2 was used (SAS Institute, Cary, NC).
Results

Comparison of murine and human bone marrow cells in the granulocyte-macrophage colony-forming unit assay. To extrapolate our in vivo data into a clinical setting, we compared the sensitivity of human and murine cells to etoposide, daunorubicin, doxorubicin, and dexrazoxane alone (Fig. 1). Murine cells were slightly more sensitive to the highest concentrations of daunorubicin and slightly less sensitive to doxorubicin than human cells; in general, human and murine cells responded similarly, though showing no species specificity (Fig. 1B and C). As expected, dexrazoxane alone did not inhibit colony formation (Fig. 1D).

Dexrazoxane protection from topoisomerase II poisons in an in vitro granulocyte-macrophage colony-forming unit assay. A concentration of poison that caused >75% inhibition of colony formation in the initial experiments was chosen for further experiments using dexrazoxane. Pretreatment of cells with dexrazoxane before 50 μmol/L etoposide offered protection over the full range of doses (Fig. 2A). Similar protection was shown using daunorubicin (Fig. 2B). However, dexrazoxane did not increase colony counts following doxorubicin treatment using either murine or human cells (Fig. 2C).

Dexrazoxane reduces etoposide and daunorubicin-mediated myelosuppression in vivo. First, mice were treated with doses of dexrazoxane from 50 to 500 mg/kg to determine toxicity and to establish a range of nonmyelotoxic doses from the drug itself. There was no myelosuppression at doses of dexrazoxane up to and including 250 mg/kg; however, at 500 mg/kg, there was a highly significant reduction in the WBC count (Fig. 3A). Platelet and RBC counts were also measured and although some significant changes occurred, these were not dose related (Fig. 3B and C). Second, we compared the ability of dexrazoxane to protect against myelosuppression induced by high doses of etoposide because we aim to use dexrazoxane in a clinical setting combined with escalated doses of etoposide. The mean nadir WBC and platelet counts following etoposide treatment were significantly increased in combination with dexrazoxane in doses from 25 to 250 mg/kg compared with 90 mg/kg etoposide alone (Fig. 4A). A similar protection was observed using 120 mg/kg etoposide (Fig. 5A). Dexrazoxane at 500 mg/kg, was less effective in all cases, this likely being due to induction of myelosuppression from the drug alone. Platelet counts were significantly increased at several concentrations of dexrazoxane (Figs. 4C and 5C). The dehydration and weight loss following treatment with etoposide alone leads to plasma concentration and dexrazoxane effectively antagonized this (Table 1), wherefore the decrease in RBC counts from the combination treatment in fact represents preservation of bodyweight and not aggravated toxicity (Figs. 4D and 5D). The toxicity of the dexrazoxane and etoposide combination to the ANC was also tested; a marked protection was observed using 90 mg/kg etoposide (Fig. 4B), and although there was an
increase in ANC from etoposide 120 mg/kg, this was not statistically significant (Fig. 5B). Recovery of the cell counts with dexrazoxane was never absolute, however: Thus, the WBC counts after etoposide + dexrazoxane were significantly different from that of saline-treated controls using both 90 and 120 mg/kg etoposide. The ANC was only obtained in the protection experiments, wherefore comparisons to saline controls were not possible.

Third, we were interested to see if the differences in responses to the three different poisons seen in the GM-CFU assay would hold true in vivo. Using daunorubicin and the dose of dexrazoxane of 125 mg/kg, which was found to be optimal for protection, the WBC and platelet were significantly different from that of saline-treated controls using both 90 and 120 mg/kg etoposide. The ANC was only obtained in the protection experiments, wherefore comparisons to saline controls were not possible.

Discussion

The use of the DNA cleavage–enhancing drugs is widespread in clinical oncology; however, dose-limiting toxicities, such as...
myelosuppression and cardiac toxicity, limit their full therapeutic exploitation. The mechanistic differentiation of DNA cleavage–enhancing drugs and catalytic inhibitors has advanced our knowledge in this area and opens up new therapeutic applications for these drugs.

In this study, we show for the first time, using both in vitro and in vivo design, that dexrazoxane works at the hematologic level to prevent damage induced by DNA cleavage–enhancing drugs. We validate the use of murine bone marrow cells by comparing their response to human cells and show similar responses in all of the scenarios studied. Using a GM-CFU in vitro assay, dose-dependent myelosuppression was obtained using etoposide, daunorubicin, and doxorubicin, and we show that dexrazoxane can protect against the antiproliferative effects of the epipodophyllotoxin etoposide and the anthracycline daunorubicin, but not against doxorubicin.

Dexrazoxane, razoxane (ICRF-159), and levrazoxane (ICRF-186) all reduced cytotoxicity from daunorubicin, but not from doxorubicin, in clonogenic assays (10, 16); similarly in vivo, razoxane rescued both mice and Syrian golden hamsters from supralethal doses of daunorubicin, but not from doxorubicin (17–19). The gastrointestinal tract was thought to be protected, which was later also suggested clinically (20, 21); however, no protection was observed on the hematologic tissue.

Likewise, dexrazoxane antagonized the antiproliferative effects of etoposide in clonogenic assays (10) and rescued mice from lethal doses of etoposide, thus allowing a 3.6-fold increase of the maximal tolerable dose of etoposide in mice (22). Our new data now show that dexrazoxane ameliorates etoposide-induced myelosuppression as well as that from daunorubicin.

Moreover, when hematologic toxicity was the pharmacodynamic end point, dexrazoxane affected doxorubicin activity quite differently than etoposide and daunorubicin, both in the in vitro and in vivo experiments. Similar differential effects were observed on weight loss.

An important consideration of coadministration of catalytic inhibitors and DNA cleavage–enhancing drugs is how it will possibly affect the anticancer treatment efficacy; there are, 

![Fig. 5. Mean nadir WBC, ANC, platelet, and RBC counts in mice treated with 120 mg/kg etoposide alone or when dexrazoxane in doses from 50 to 500 mg/kg was administered 20 minutes before etoposide. Drug doses (mg/kg), the recoveries of cell counts after etoposide and dexrazoxane as percentages of saline treated controls (%), and the numbers of mice (n) are shown. The results of comparing etoposide alone versus etoposide and dexrazoxane are denoted by stars: *, P < 0.05; **, P < 0.01; and ***, P < 0.001; ns., not statistically significant (P > 0.05). Bars, SE. A, WBC; B, ANC; C, platelet; D, RBC.](https://www.aacrjournals.org/cancerres/article-pdf/11/10/3920/4211275/3920.pdf)
however, important differences between these two processes, which suggest that a reduction in side effects does not necessarily go hand-in-hand with a reduction in the antitumor effects.

Dexrazoxane ameliorated intestinal cell damage in mice (23) and prevented skin ulceration following experimental extravasation (24) from several different anthracyclines: The mechanism of action on the prevention of skin wounds are

Table 1. The effect of dexrazoxane on weight loss from etoposide

<table>
<thead>
<tr>
<th>Dose of etoposide (mg/kg)</th>
<th>No dexrazoxane (etoposide alone)</th>
<th>Etoposide + dexrazoxane (in doses from 25 to 500 mg/kg)</th>
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<tr>
<td></td>
<td></td>
<td>25 mg/kg</td>
</tr>
<tr>
<td>90</td>
<td>0.85 ± 0.067</td>
<td>0.96 ± 0.093*</td>
</tr>
<tr>
<td>120</td>
<td>0.83 ± 0.09</td>
<td>ND</td>
</tr>
</tbody>
</table>

NOTE: The mean relative weight at nadir (the weight on nadir divided by the pretreatment weight) ± SD is shown; thus, a relative weight on nadir of, e.g., 0.90 signifies a weight loss of 10%. Weight loss from etoposide + dexrazoxane was compared with etoposide alone.
Abbreviation: ND, not done.
*P < 0.001.
†P < 0.05.

Dexrazoxane ameliorated intestinal cell damage in mice (23) and prevented skin ulceration following experimental extravasation (24) from several different anthracyclines: The mechanism of action on the prevention of skin wounds are

Fig. 6. Mean nadir WBC, ANC, platelet, and RBC counts in mice treated with 7.5, 15, and 30 mg/kg daunorubicin (Dau) alone or when dexrazoxane, 125 mg/kg, was administered 20 minutes before daunorubicin. Drug doses (mg/kg), the recoveries of cell counts after daunorubicin and dexrazoxane as percentages of saline treated controls (%), and the numbers of mice (n) are shown. The results of comparing daunorubicin alone versus daunorubicin and dexrazoxane are denoted by stars: ★, P < 0.05; ★★, P < 0.01; and ★★★, P < 0.001 ns., not statistically significant (P > 0.05). Bars, SE. A, WBC; B, ANC; C, platelet; D, RBC.
unclear, whereas the current understanding is that dexrazoxane protects the heart (6) from iron-mediated oxidative damage through the iron-chelating properties of the ring-opened molecule rather than through its topoisomerase inhibitory properties (4, 6, 8, 9).

Importantly, myelosuppression clinically from the coadministration of dexrazoxane and doxorubicin is slightly aggravated compared with doxorubicin alone (25–28), whereas the effect on myelosuppression from daunorubicin is not as well elucidated: However, in one report, it seemed to be unaffected (29).

Although most researchers agree that a negative interference of dexrazoxane on the anthracycline anticancer efficacy has never been documented, it needs consideration that most of the clinical data were generated from protocols administering doxorubicin (6, 8), not daunorubicin.

It is intriguing why dexrazoxane reduces the etoposide- and daunorubicin-induced weight loss, myelosuppression, and antiproliferative effects in the GM-CFU assays, but not from doxorubicin. One explanation is the stronger DNA binding of doxorubicin compared with daunorubicin (30); this results in prolonged cellular retention (31), possibly leading to more of unopposed drug activity following the cellular clearance of dexrazoxane compared with daunorubicin that is cleared faster (31).

That the anticancer effect from doxorubicin, which is thought to be mainly mediated by topoisomerase II, is unaffected by the catalytic inhibitor dexrazoxane is perhaps explained by its prolonged cellular retention. Increased cellular retention might reflect the fact that doxorubicin activity is the same following single dose versus fractionated administration, whereas daunorubicin must be administered in a fractionated schedule (days 1-2-3) for optimal efficacy.

Etoposide activity is thought to be mediated primarily through topoisomerase II (1, 2, 14, 32), whereas a plethora of mechanisms (e.g., free radicals, DNA intercalation, and adduct formation) as well as direct induction of apoptosis have been suggested for the anthracyclines, in addition of course to its effect on topoisomerase II (1, 3, 4, 15). How the different cytotoxic routes confer anticancer activity and unwanted
Table 2. The effect of dexrazoxane on weight loss from daunorubicin and doxorubicin

<table>
<thead>
<tr>
<th></th>
<th>Daunorubicin (in doses from 7.5 to 30 mg/kg)</th>
<th>Doxorubicin (in doses from 10 to 20 mg/kg)</th>
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<tbody>
<tr>
<td></td>
<td>7.5 mg/kg</td>
<td>15 mg/kg</td>
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<tr>
<td>Daunorubicin alone (no dexrazoxane)</td>
<td>0.95 ± 0.023</td>
<td>0.95 ± 0.024</td>
</tr>
<tr>
<td>Daunorubicin + dexrazoxane 125 mg/kg</td>
<td>0.94 ± 0.032 ns.</td>
<td>0.94 ± 0.018 ns.</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
<td>15 mg/kg</td>
</tr>
<tr>
<td>Doxorubicin alone (no dexrazoxane)</td>
<td>0.94 ± 0.02</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>Doxorubicin + dexrazoxane 125 mg/kg</td>
<td>0.93 ± 0.028 ns.</td>
<td>0.93 ± 0.04 ns.</td>
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NOTE: The mean relative weight on nadir (the weight on nadir divided by the pretreatment weight) ± SD is shown; thus, a relative weight on nadir of, e.g., 0.95 signifies a weight loss of 5%. Weight loss from cytotoxics and dexrazoxane was compared with cytotoxics alone.

Abbreviation: ns., not statistically significant (P > 0.05).

*P < 0.001.

toxicity, respectively, is unknown. Thus, both the cardiotoxicity and the experimental skin ulceration from different anthracyclines were potently ameliorated by dexrazoxane, indicating shared mechanisms of actions.

Clinical trials have shown that dexrazoxane neither reduces hematologic toxicity nor the antitumor activity of doxorubicin and our findings certainly corroborate this observation. On the other hand, a possible antagonism of dexrazoxane on the antitumor efficacy can only be addressed in specific daunorubicin clinical trials.

In mice with brain tumor, coadministration of supralethal doses of etoposide and dexrazoxane significantly increased treatment efficacy and survival, compared with equitoxic doses of etoposide administered alone. This is because the lipophilic drug etoposide passes the blood-brain barrier to a much greater extent than the hydrophilic drug dexrazoxane (13, 22). This brain-tumor–targeted anticancer treatment and simultaneous extracerebral protection against toxicity is the objective of ongoing clinical trials, assessing if dexrazoxane and escalated doses of etoposide can be safely coadministered. Our new data indicate that etoposide dose escalation in combination with dexrazoxane with an accompanying amelioration of hematoxicity is possible. The concept of providing extracerebral DNA damage protection represents a unique new way of attacking the unwanted toxicity from conventional cytotoxic chemotherapy, and, if successful, will allow the safe use of increased drug doses for the benefit of future cancer patients, e.g., with brain metastases.

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References


Dexrazoxane Protects against Myelosuppression from the DNA Cleavage–Enhancing Drugs Etoposide and Daunorubicin but not Doxorubicin
