Dose-Response Relationship of Dexrazoxane for Prevention of Doxorubicin-induced Cardiotoxicity in Mice, Rats, and Dogs

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ABSTRACT

Dexrazoxane (DZR), ADR 529, ICRF-187 ameliorates doxorubicin (DOX)-induced cardiotoxicity in animals, and is recommended as a cardioprotective agent in patients receiving cumulative doses of DOX above 300 mg/m². A DZR:DOX dose ratio of 10:1 is recommended based on studies in patients receiving 50 mg/m². Since DOX may be used at much higher doses in certain clinical settings, we evaluated the ability of DZR to protect against cardiomyopathy in animals given bolus doses of DOX at varying dose levels. The severity and extent of the cardiomyopathy were evaluated historically and expressed as the mean total score (MTS). Mice were given 10 doses of DOX (2 or 4 mg/kg) over a 7-week period. Without DZR, the MTS 4 weeks after the last treatment was 3.7 with 4 mg/kg DOX and 1.3 with 2 mg/kg DOX. DZR at 5:1, 10:1, and 20:1 dose ratios caused a dose-dependent decrease in the MTS but was less efficacious with the higher, more cardiotoxic dose of DOX. Rats were given DOX at 0.2, 0.4, and 0.8 mg/kg with a 20:1 ratio of DZR weekly for 13 weeks. Cardiomyopathy was most severe with the highest dose of DOX in the absence of DZR, especially in males, and progressed during the 6 weeks following the last treatment. DZR reduced the MTS in both sexes but in the males given the highest dose of DOX, there was still a significant amount of cardiac damage compared to vehicle-treated controls. Dogs were given 0.1, 0.3, and 0.8 mg/kg/DOX with 20:1 ratio of DZR weekly for 13 weeks. DZR reduced the MTS significantly (P < 0.05) in males and females but cardiac lesions were still present in each of the DZR-treated dogs. The results indicate that although DZR is highly effective in attenuating the cardiomyopathy caused by DOX, dose ratios of DZR:DOX capable of providing total or nearly complete cardioprotection at low doses of DOX are less efficacious at higher doses of DOX. One possible explanation for this effect is the marked pharmacokinetic difference between DZR and DOX, with DZR undergoing a much more rapid rate of elimination from the body compared to DOX. These findings point to the need for further studies to optimize the dose scheduling of DZR before using it clinically with bolus doses of DOX above those currently recommended.

INTRODUCTION

The cardiotoxic effects of DOX, one of the most widely used and effective drugs for the treatment of cancer, have been recognized for many years (1, 2). Although evidence of cardiotoxicity can be detected in adults receiving cumulative doses of DOX of <300 mg/m² (3), there is a marked increase in the risk of clinical cardiotoxicity at cumulative doses above 550 mg/m², which may lead to CHF and death (1, 4). In children, cumulative doses of 210 mg/m² DOX or cumulative doses above 550 mg/m² which may lead to CHF and death (1, 4). In children, cumulative doses of 210 mg/m² DOX or more may cause adverse effects on the heart, which may result in severely compromised cardiac function several years later (5, 6).

DZR, a bis-dioxopiperazine derivative, which is hydrolyzed to a ring-opened chelating agent, was evaluated as an antineoplastic agent by Creighton et al. (7) nearly 30 years ago. It was subsequently found to ameliorate the cardiotoxic effects of DOX in several animal species (8–10). These studies served as the basis for the clinical evaluation of DZR as a cardioprotective agent in patients receiving DOX for the treatment of breast cancer (3, 11). DZR was found to be highly effective in reducing the severity and the incidence of DOX-induced cardiotoxicity in these clinical trials, and is now marketed in a number of countries as a cardioprotective drug in conjunction with DOX therapy.

Although the cardioprotective efficacy of DZR has been studied extensively only in patients with breast cancer receiving doses of 50 mg/m² DOX every 3 weeks, there may be interest in using it in other clinical settings such as pediatric sarcoma (12) or with chemotherapy regimens using much higher bolus doses of DOX (13, 14).

It had been reported that the cardioprotective effect of DZR was dose-dependent at DZR:DOX ratios from 4:1 to 20:1 in mice given 10 doses of 4 mg/kg DOX (15) and from 5:1 to 20:1 in rats given weekly doses of 1 mg/kg DOX (16). In humans, ratios of 10:1 and 20:1 gave significant cardioprotection with doses of 50 mg/m² DOX (3, 11). However, none of these studies demonstrated complete cardioprotection, suggesting that additional studies aimed at dosage optimization may be worthwhile, especially if higher doses of DOX are contemplated.

The present studies were conducted to evaluate the cardioprotective effect of DZR in animals given varying doses of DOX.

MATERIALS AND METHODS

DZR and DOX were manufactured by Farmitalia Carlo Erba (now Pharmacia and Upjohn; Milan, Italy). The DZR was provided as the lyophilized HCl salt and was reconstituted in 0.167 M sodium lactate at a concentration of 10 mg/ml. The reconstituted solution was stable at room temperature for up to 6 h and under refrigeration for up to 24 h, and the drug was used within these time limits. DOX was prepared fresh daily by dissolving it in saline at 2 mg/ml.

Mouse Studies. Two studies were conducted in the mouse. The first (study 1) compared the DOX-induced cardiotoxicity at 4 weeks and 15 weeks after the last of 10 doses of 4 mg/kg DOX in female ICR Swiss mice weighing 18–20 g. They were caged individually in suspended stainless steel cages and kept in a room with controlled temperature and humidity and a 12-h light/dark cycle. The mice had free access to Purina Mouse Chow and water throughout the study.

Twenty-six mice were given 4 mg/kg DOX and 26 controls were given saline i.v. into a lateral tail vein in a volume of 10 ml/kg according to the dosage schedule described by Bertazzoli et al. (17), i.e., twice weekly for weeks 1, 2, and 5–7. At week 11, 13 controls and 13 DOX-treated mice selected before the treatment began were sacrificed by CO₂ asphyxiation. The remaining mice were sacrificed in the same way 11 weeks later, 15 weeks posttreatment.

The heart was quickly removed and fixed in 4% buffered paraformaldehyde. Penetration of the fixative into the tissue was facilitated by gentle compression of the heart to expel blood and air from the chambers. Forty-eight h later, the heart was removed, dehydrated in ethanol, infiltrated, and embedded in paraffin. Two-μm sections were stained with toluidine blue and examined microscopically in a random order by a pathologist without prior knowledge of treatment assignment. The hearts were scored on a scale of 0–4, considering both the severity and extent of pathology: grade 0, normal histo-
logical appearance; grade 1, very slight; scattered, single myocardial fibers with vacuolation or degenerative changes; grade 2, slight; scattered small groups of altered myocardial fibers throughout the atrial and ventricular myocardium; grade 3, moderate; disseminated myocardial fiber vacuolation or degeneration with only occasional focal unaffected areas; and grade 4, marked; confluent groups of affected myocardial fibers; most myocardial fibers affected.

The second mouse study (study 2) was conducted to determine the effect of increasing dose ratios of DZR:DOX on the cardiomyopathy induced by 10 doses of DOX at 2 and 4 mg/kg. Female Crl:CD-1(ICR)BR mice supplied by Charles River Italia (Calco, Como, Italy), approximately 5 weeks old and weighing 22—30 g, were used.

The mice were kept in groups of three in cages with sawdust bedding in a room with controlled temperature (19—23°C) and humidity (42—68%) and a 12-h light/dark schedule. They were allowed free access to water and 4RF21 GLP pelleted mouse food supplied by Mucedola S.r.l. (Milan, Italy).

The mice were assigned randomly (15/group) to one of nine groups: group 1, lactate/saline control; groups 2—5 received 0, 10, 20, and 40 mg/kg DZR, respectively, 30 min before 2 mg/kg DOX; groups 6—9 received 0, 40, 80, and 120 mg/kg DZR 30 min before 4 mg/kg DOX. The vehicles and drugs were administered i.v. using the schedule described for study 1. In study 2, all of the mice were sacrificed 4 weeks after the last dose by i.p. administration of sodium thiopental. The hearts were fixed and embedded in polymethylacrylate as described above but were sectioned at 1 μm.

The histopathological evaluation of the hearts from study 2 was performed using a scoring system described by Solcia et al. (18), in which the cardiomyopathy is expressed as a product of the severity and the extent of the damage.

Severity was defined as: grade 1, sarcoplasmic microvacuolations and/or inclusions, cellular edema, or interstitial edema; and grade 2, as in grade 1 plus sarcoplasmic macrovacuolations or atrophy, necrosis, fibrosis, endocardial lesions, and thrombi. Extent was defined as: grade 0.5, less than 10 altered myocytes/field; grade 1, single altered myocytes; grade 2, scattered small groups of altered myocytes; grade 3, several small groups of altered myocytes; grade 4, groups of altered and confluent myocytes; and grade 5, most myocytes affected.

The MTS = Σ(S × E)/number of animals. The severity and the extent of the cardiomyopathy were evaluated separately and were found to vary in parallel among the treatment groups. Therefore, only the MTS data were used to compare treatment effects.

**Rat Study.** Male and female Sprague Dawley (Crl:CD(SD)BR) rats weighing 200—240 g and 130—170 g, respectively, were obtained from Charles River Breeding Laboratories (Portage, MI). They were housed singly in suspended wire mesh cages in a room with a 12-h light/dark cycle and controlled temperature (21—23°C) and relative humidity (40—50%). The rats had free access to tap water and Purina Laboratory Rat Chow throughout the study. They were randomly assigned to groups of 21/rat/treatment based on body weight. Fifteen rats per sex per group were selected prior to treatment to be sacrificed 1 week after the last treatment, and the remaining six rats per group were held for an additional 5 weeks. The treatments were as follows: group 1, sodium lactate/saline control; group 2, 16 mg/kg DZR plus saline; group 3, 4 mg/kg DZR plus 0.2 mg/kg DOX; group 4, 8 mg/kg DZR plus 0.4 mg/kg DOX; group 5, 16 mg/kg DZR plus 0.8 mg/kg DOX; and group 6, sodium lactate plus 0.8 mg/kg DOX.

The rats were dosed by slow i.v. injection into a tail vein once weekly for 13 weeks; either DZR or sodium lactate was administered about 30 min before DOX or saline. The rats were sacrificed by exsanguination following sodium pentobarbital anesthesia. The heart was fixed and embedded in polymethylacrylate as described for the mice. The severity of the cardiomyopathy, consisting of vacuolar degeneration of myofibrils, was scored as described above (study 1).

**Dog Study.** Male and female beagle dogs (10—12 months old) were housed individually in stainless steel cages in rooms maintained between 20 and 27°C and between 30 and 70% relative humidity. Except for those days when the dogs were dosed, they were allowed access to food for 2 h each morning. On days of dosing, food was available from the time dosing was completed until the following morning. Water was available ad libitum.

The dogs were randomized to treatment groups by a computerized system to maintain comparable mean group body weights. Each group consisted of six males and six females, with four per sex designated to be sacrificed 1 week after the last treatment and the remaining dogs 6 weeks posttreatment. The treatments, once weekly for 13 weeks, were as follows: group 1, sodium lactate/saline; group 2, 16 mg/kg DZR plus saline; group 3, 2 mg/kg DZR plus 0.1 mg/kg DOX; group 4, 6 mg/kg DZR plus 0.3 mg/kg DOX; group 5, 16 mg/kg DZR plus 0.8 mg/kg DOX; and group 6, sodium lactate plus 0.8 mg/kg DOX.

The drugs were administered through an i.v. catheter into the cephalic or saphenous veins. The DZR and the sodium lactate solutions were infused at approximately 4 ml/min, 30 min before the DOX or saline. A syringe pump was used for volumes greater than 10 ml. With the exception of the initial treatment when they were administered at a rate of about 4 ml/min, the DOX and saline solutions were given at a rate of 2 ml/min. The catheter was then flushed with 3 ml saline containing 20 units heparin/ml. The dogs were sacrificed at the scheduled times by exsanguination following phenobarbital anesthesia. The heart was fixed in neutral buffered formalin, embedded in polymethylacrylate, sectioned at 2 μm, and stained with toluidine blue. The sections were scored as described for study 1.

**Statistical Analyses.** In study 2, the cardiotoxicity induced by DOX alone and the DOX/DZR combinations was analyzed using the Kruskal-Wallis test (19) followed by the Dunn one-tailed multiple comparison test (20) to compare the MTS of treated groups with the control group and of the DZR-treated groups with the DOX-treated groups. The Wilcoxon one-tailed test (21) was used to compare the different ratios of DZR:DOX. The incidence of slight/moderate cardiomyopathy in study 2 mice was analyzed using the χ² test and for the rat and dog studies, the significance of differences between groups was determined using the Mann-Whitney U test (StatMost for Windows, Version 3.00, DataMost, Salt Lake City, UT).

**RESULTS**

**Mice.**

**Study 1.** The initial study demonstrated that, as previously reported (17), 10 doses of 4 mg/kg DOX over a 7-week period caused cardiomyopathy in all mice examined 4 weeks after the last dose (MTS ± SD, 2.3 ± 0.5, n = 11). At this point, there were no signs of CHF, e.g., ascites, severe edema, or pleural effusion. The lesions in hearts examined 15 weeks after the last dose were only slightly more severe (2.9 ± 0.8, n = 9), but 4 of 13 mice died between 4 and 15 weeks after treatment with signs of CHF, and 5 of the 9 survivors exhibited CHF.

**Study 2.** Two mice given 4 mg/kg DOX alone died at the end of the 4-week posttreatment period and one mouse given 4 mg/kg DOX with the 10:1 DZR treatment died 5 days after the last treatment in poor physical condition attributed to drug treatment. Five deaths occurred due to technical problems during handling or dosing of the mice. The effects of DZR on the cardiomyopathy induced by 2- and 4-mg/kg doses of DOX are shown in Fig. 1. In the absence of DZR, the MTS was 3.7 with 4 mg/kg DOX and 1.3 with 2 mg/kg DOX (P < 0.01) compared to control. The incidence of slight/moderate cardiomyopathy (MTS ≥ 2) was 13 of 13 and 6 of 15 mice, respectively (P < 0.01; Table 1).

DZR decreased the MTS at dose ratios of 5:1, 10:1, and 20:1 with 2 mg/kg DOX; however, the effect was statistically significant only at the highest ratio when compared to the MTS for the mice given 2 mg/kg DOX alone (P < 0.01). The incidence of slight/moderate cardiomyopathy also decreased from 6 of 15 in the 2 mg/kg DOX controls to 4 of 15, 2 of 15, and 1 of 14 at the 5:1, 10:1, and 20:1 dose ratios, respectively.

In mice given the 4 mg/kg DOX, DZR decreased the MTS at 10:1 (P < 0.05), 20:1 (P < 0.01), and 30:1 (P < 0.01). However, unlike the effects observed with the 2-mg/kg dose of DOX, the MTS in the mice given the 10:1 dose ratio of DZR with the 4-mg/kg dose of DOX was higher than that in the vehicle-treated controls (P < 0.01), and 6
of 12 mice had slight/moderate cardiomyopathy. There was a mild degree of cardiomyopathy in the mice given 4 mg/kg DOX with the 20:1 and 30:1 ratios, and slight/moderate cardiomyopathy was present in 4 of 14 and 1 of 15 mice, respectively.

Rats

DOX doses of 0.2, 0.4, and 0.8 mg/kg given weekly for 13 weeks with DZR at a ratio of 20:1 were more cardiotoxic in males than in females (Table 2). This was especially noticeable in group 6 animals in which there was also a marked progression in the severity of the cardiomyopathy from weeks 14–19. There was no evidence of cardiomyopathy in either the males given the lowest dose of DOX or in the females given the low or middle dose of DOX. It is not known whether DZR was completely cardioprotective in these low and middle dose groups, since groups given DOX alone at these lower doses were not included. At the highest dose of DOX, DZR reduced the severity of the DOX-induced cardiotoxicity at 14 (P < 0.01) and 19 weeks (P < 0.05). In addition, the number of female rats with measurable cardiac damage was decreased by DZR to 4 of 15 and 2 of 6, respectively.

Table 1 Incidence of slight/moderate cardiomyopathy (score ≥ 2) in mice given DZR with either 2 or 4 mg/kg DOX

<table>
<thead>
<tr>
<th>DZR:DOX*</th>
<th>DOX (mg/kg)</th>
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<tr>
<td>DOX alone</td>
<td>6/13b</td>
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<td>10:1</td>
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<td>20:1</td>
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<td>30:1</td>
<td>NT</td>
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* Treatments given i.v. on days 1, 4, 8, 11, 29, 32, 36, 39, 43, and 46. Necropsies were performed on day 71.

DISCUSSION

The initial findings by Herman and Ferrans (8) regarding the amelioration of anthracycline-induced cardiotoxicity by DZR have been corroborated by many investigators in laboratory animals and humans (22). The present studies demonstrate that the cardioprotective effects of DZR in DOX-treated animals are dependent on both the DZR:DOX ratio and the dose of DOX. Thus, DZR:DOX ratios, which provided essentially complete cardioprotection at low doses of DOX, were less effective in preventing the severe cardiomyopathy caused by higher doses of DOX.
A recent report by Della Torre et al. (23) suggested that DZR not only provided a significant dose-dependent cardioprotection in male rats given 1 mg/kg DOX weekly for 7 weeks (total cumulative dose of 7 mg/kg), but that there was a trend for DZR to decrease the severity of cardiomyopathy from the time of peak cardiotoxicity, 5 weeks after the last dose of DOX, to the end of the study, 28 weeks after the last dose of DOX. Herman and Ferrans (24) also reported an apparent reversal of daunorubicin-induced cardiomyopathy in rabbits treated with DZR. In the present rat study, in which hearts were examined at 1 week and 6 weeks after the last dose of DOX, DZR appeared to halt or attenuate the progression of the cardiac damage in the males given the middle dose (5.2 mg/kg total dose of DOX) and in the females given the high dose (10.4 mg/kg total dose of DOX) where there was mild cardiomyopathy (MTS, 0.33). In males that received the highest dose of DOX, the cardiomyopathy was more severe than in the females, and although there was a decrease in the MTS with DZR treatment, there was still a significant amount of cardiac damage compared to vehicle-treated controls. There was also a tendency for the cardiomyopathy to progress during the 6 weeks after treatment. Thus, even though cardiomyopathy caused by high doses of DOX is considered to be progressive and irreversible (25), it is possible that cardiac myocytes are able to recover from mild damage. These findings point to the importance of optimizing the dose and scheduling of DZR therapy to provide maximum cardioprotection when high bolus doses of DOX are used.

The results from the rat studies also indicate that DOX causes more severe cardiomyopathy in males than in females. This is in contrast to the findings in DOX-treated children in whom the risk of cardiotoxicity was higher in females (25). The reason for this gender-related difference in either rats or humans is not clear, but additional studies to explain these results may prove helpful in elucidating the mechanism responsible for anthracycline-induced cardiotoxicity.

Although the concept of expressing the dose of DZR as a multiple of the dose of DOX implies some type of stoichiometric relationship between the two drugs and is convenient from the standpoint of dosage administration, the present results suggest that this relationship applies only over a limited range of DOX doses. For example, the highest bolus doses of DOX used in the present studies, expressed in terms of body surface area, were 12 mg/m², 4.8 mg/m², and 16 mg/m² in the mice, rats, and dogs, respectively, which are considerably less than the 50-mg/m² bolus dose of DOX used in the clinical trials on DZR (3, 11). Yet, in the mice and rats, a 2-fold increase, and in dogs, a 2.7-fold increase in the dose of DOX resulted in a marked reduction in the efficacy of DZR when the DZR:DOX ratio was held constant. One explanation for this is that more than one mechanism is responsible for the DOX-induced cardiotoxicity, and that DZR is effective only against one of these mechanisms. For example, it has been proposed by Myers et al. (26) that anthracyclines cause cardiotoxicity through the formation of free radicals. The free radicals may be generated by both iron-dependent and -independent mechanisms (27, 28). Since DZR probably acts as a metal ion chelator following ring opening (29), it would be expected to be effective in blocking the formation of free radicals formed through iron-dependent mechanisms but not those formed enzymatically. Yet, except for the magnitude of the injury, the DOX-induced cardiomyopathy would appear to be microscopically similar with or without DZR, because in both instances the lesions are caused by free radicals. If this was the case, it would be nearly impossible to achieve complete cardioprotection by increasing the dose of DZR with higher doses of DOX. Indeed, the results of the present studies lend support to this hypothesis, since the cardiac lesions were histologically similar in the presence and absence of DZR and increasing the DZR:DOX ratio to 20:1 in the mice given 2 mg/kg DOX or to 30:1 in those given 4 mg/kg DOX did not provide complete cardioprotection.

Alternatively, the inability of DZR to provide complete cardioprotection at high doses of DOX may be related to either pharmacokinetic differences or dose-dependent interactions between the two drugs. In humans, for example, the terminal half-lives of DOX and DZR are 39.5 h and 4.16 h, respectively (30). In rats and dogs also, the terminal half-life of DZR is considerably shorter than that of DOX (31, 32). Thus, plasma levels following a single dose of DZR may not be high enough over a sufficient period of time to provide adequate protection against a high bolus dose of DOX. Plasma levels of DZR and DOX alone and in combination suggested that there was no interaction between these drugs at a ratio of 20:1 in either dogs (32) or humans (30); however, there may be dose-dependent effects on the relative uptake of DOX and DZR at the level of the cardiomyocyte. Villani et al. (33) have reported that doses of 125 mg/kg DZR reduced but did not eliminate cardiomyopathy in rats given 3 mg/kg DOX, a 42:1 dose ratio. In this study, DZR caused an unexpectedly highly significant increase in the uptake of DOX by the myocardium. In view of the results of the present studies, it would be of interest to examine the effects of DZR on myocardial uptake of DOX at lower, less effective cardioprotective dose ratios.

If pharmacokinetic differences are responsible, at least in part, for the reduced efficacy of DZR with higher doses of DOX seen in these studies, then alternative dosing schedules should be investigated in animals before using DZR in high-dose DOX regimens in humans. For example, a total dose of DZR equivalent to 10 times the bolus dose of DOX could be given in divided doses over a period of several hours following the administration of DOX to provide higher circulating levels of DZR during the latter phases of DOX elimination from the body. The individual doses of DZR might be varied so as to mimic the plasma levels of DOX following bolus administration; e.g., 5:1 DZR:DOX given at the same time as DOX, followed by 3:1 and 2:1 dose equivalents at 6 and 12 h later without DOX. If very high DZR:DOX ratios promote the myocardial uptake of DOX as was observed in the study by Villani et al. (33), this dosing schedule in which the highest DZR:DOX ratio is 5:1 should minimize the uptake of DOX by the myocardium.
Our results confirm the cardioprotective activity of DZR in animals but suggest that the degree of protection depends on the bolus dose of DOX. DZR:DOX dose ratios which provide essentially complete cardioprotection with low doses of DOX are less effective when given with higher, more severe cardiotoxic doses of DOX. These findings point to the need for additional studies to optimize the dose scheduling of DZR before using this drug clinically with higher than currently recommended doses of DOX.

REFERENCES

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