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ORIGINAL ARTICLE

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Comparison of the protective effects of amifostine and dexrazoxane against the toxicity of doxorubicin in spontaneously hypertensive rats

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Abstract *Purpose*: To compare the protective effects of amifostine and dexrazoxane against the chronic toxicity induced by doxorubicin in spontaneously hypertensive rats (SHR). *Methods*: The animals were pretreated with amifostine (200 mg/kg, i.p.), dexrazoxane (25 mg/kg, i.p.) or saline 30 min before the administration of doxorubicin (1 mg/kg, i.v.), once-weekly for 12 weeks. Control animals received similar amounts of amifostine or saline. The SHR underwent necropsy examination 1 week after the last dosing, and cardiac, renal, and gastrointestinal lesions were graded semiquantitatively. *Results*: Amifostine and dexrazoxane provided equal degrees of protection against the renal toxicity of doxorubicin. However, dexrazoxane was more cardioprotective than amifostine, and prevented the mortality induced by doxorubicin. This mortality was not decreased by pretreatment with amifostine. The loss of body weight caused by doxorubicin was actually worsened by coadministration of amifostine. *Conclusions*: Compared to dexrazoxane, amifostine provided a comparable degree of protection against the nephrotoxicity of doxorubicin, but was less cardioprotective and did not prevent the mortality and loss of body weight produced by doxorubicin. These differences may be related to the fact that amifostine may act as a scavenger of reactive oxygen species, whereas dexrazoxane may prevent their formation.

Key words Doxorubicin · Cardiomyopathy · Nephrotoxicity · Amifostine · Dexrazoxane

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Introduction

Chronic cardiotoxicity, manifested as cardiomyopathy, continues to pose a serious limitation to the optimal clinical use of doxorubicin and other anthracycline antineoplastic agents [23, 36]. The pathogenesis of this cardiomyopathy is thought to be related to multiple factors, the most important of which appears to be the formation of cytotoxic reactive oxygen species (ROS) [13, 26, 29]. There is evidence to indicate that doxorubicin and iron form a complex that is capable of reacting with oxygen to generate superoxide and hydroxyl radicals [16, 26]. Attempts to protect the myocardium from this toxicity have included the administration of compounds that interfere with the formation of, or the effects of, ROS.

Dexrazoxane (ICRF-187), an intracellular iron chelator, has been shown to reduce the formation of ROS induced by the doxorubicin-iron complex and to decrease the chronic cardiotoxic effects of doxorubicin in a number of animal species [17] and in patients [32]. Because oxidative mechanisms have been implicated in the pathogenesis of doxorubicin cardiotoxicity, thiols and other types of ROS scavengers have been examined as potential cardioprotectors. One of these compounds, *N*-acetylcysteine, which ameliorates acute high-dose doxorubicin cardiotoxicity [8], has not been shown to decrease chronic cardiotoxicity in either experimental [20] or clinical studies [10]. Amifostine (*S*-2-(3-aminopropylamino) ethylphosphorothioic acid; WR2721), a sulfhydryl-containing radioprotective agent, has been found to exert protective activity against the bone marrow suppression, nephrotoxicity and neurotoxicity associated with alkylating agents and platinum-based compounds [15, 31, 33]. It has been suggested that, because of its inherent antioxidant activity, amifostine might also be useful in protecting the heart against the toxic effects of doxorubicin. At the present time, there is only limited information regarding the potential cardioprotective activity of amifostine.

Both amifostine and WR1065 (the dephosphorylated metabolite of amifostine) increase the viability of neonatal rat cardiac myocytes exposed to doxorubicin [9]. In vivo studies have shown that both of these agents are capable of reducing certain indices of doxorubicin-induced cardiotoxicity [3, 7]. In the present study the protective effects of dexrazoxane and amifostine against the toxic effects of doxorubicin in spontaneous hypertensive rats (SHR) were compared. As described in detail previously, these animals have become a well-established model for studies of chronic anthracycline cardiotoxicity [19, 21].

Materials and methods

The experimental animals comprised 66 12-week-old male SHR of the Aoki-Okamoto strain obtained from Charles River Breeding Laboratories (Wilmington, Mass.). All animals were given rodent chow and water ad libitum. The experiment commenced after a 2-week acclimation period. All procedures involving animals were in compliance with the Guidelines for the Use and Care of Laboratory Animals (NIH Publication 85-23) and were in accord with standard operating procedures approved by the Institutional Animal Care and Use Committee, Center for Drug Evaluation and Research, Food and Drug Administration.

The SHR were divided into five groups. Group 1 (18 animals) received 12 weekly injections of 1 mg/kg doxorubicin via a tail vein. Group 2 (13 animals) received 200 mg/kg amifostine i.p. weekly for 12 weeks 30 min prior to doxorubicin. Group 3 (18 animals) received 25 mg/kg dexrazoxane i.p. weekly for 12 weeks 30 min prior to doxorubicin. Group 4 (5 animals) received 200 mg/kg amifostine i.p. followed by saline i.v. (0.1 ml/100 g body weight). Group 5 (12 animals) served as a control group and received weekly i.p. and i.v. injections of normal saline for 12 weeks. Amifostine (obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.), dexrazoxane and lyophilized doxorubicin (obtained from Pharmacia Laboratories, Columbus, Ohio) were dissolved in normal saline just prior to use and injected in dose volumes of 0.4 ml/100 g body weight (50 mg/ml), 0.25 ml/100 g body weight (10 mg/ml) and 0.1 ml/100 g body weight (1 mg/ml), respectively. Each animal was observed daily and weighed weekly. The study was terminated 1 week after the 12th treatment period (13 weeks).

Blood samples were collected for clinical chemistry determinations (Maryland Medical Metpath, Baltimore, Md.). Animals were euthanized with an overdose of sodium pentobarbital. The heart, one kidney and portions of liver, lungs and small intestine were excised from each animal and fixed in 10% neutral buffered formalin. The hearts were embedded in glycol methacrylate resin. Sections of the plastic-embedded myocardial tissue (1 μm thick) were stained with hematoxylin-eosin or alkaline toluidine blue. All other tissues were embedded in paraffin and stained with hematoxylin-eosin.

The frequency and severity of doxorubicin-induced cardiac alterations were assessed by light microscopic examination of the plastic sections according to the semiquantitative method of Billingham [4]. This grading system is based on the percentage of myocytes showing myofibrillar loss and cytoplasmic vacuolization: 0 = no damage, 1 = < 5%, 1.5 = 5–15%, 2 = 16–25%, 2.5 = 26–35% and 3 = > 35%. Doxorubicin-induced renal alterations were evaluated as described previously [19]. The features of this nephropathy include glomerular vacuolization and sclerosis, marked atrophy and dilatation of the tubules, protein casts in the tubular lumina, and interstitial lymphocytic infiltration. The severity of these alterations was assessed semiquantitatively on a scale of 0 to 4, in which 0 = no alterations, 1 = minimal, 2 = mild, 3 = moderate and 4 = severe.

All sections were evaluated without prior knowledge of the treatment given to the animals. A Mann-Whitney test was utilized to determine the significance of the differences in the severity of the cardiomyopathy and nephropathy scores among the various treatment groups. The Tukey-Kramer multiple comparisons test was used to assess differences in body weights between the groups. For both tests, $P < 0.05$ was used as the level of significance.

Results

General toxicity and weight changes

Data on the mortality and changes in body weights during the course of the study are summarized in Table 1. Of the 49 doxorubicin-treated SHR, 29 died prior to termination of the experiment. The majority (14 of 18) of these deaths occurred in the groups given doxorubicin alone and the combination of amifostine and doxorubicin (13 of 13). Most deaths occurred after the 11th or 12th dose of doxorubicin or after the 9th to 11th doses of amifostine plus doxorubicin. In contrast, only two deaths (one after seven doses and one after nine doses) occurred in the group of 18 SHR given dexrazoxane and doxorubicin. There were no deaths in the group of 5 animals receiving amifostine alone or in the saline-treated control group of 12 animals.

At the end of the 12-week experimental period, two of five SHR given amifostine alone had decreased serum levels of calcium (4.6 and 4.2 mg/dl) compared to the saline control SHR (9.2–9.3 mg/dl). Decreases in serum calcium concentration were also detected in the three SHR that survived all 12 doses of doxorubicin (5.8, 6.5 and 8.1 mg/dl). However, due to the 100% mortality, blood samples were not available from SHR given amifostine plus doxorubicin.

Table 1 Mortality and body weights of SHR given doxorubicin (1 mg/kg) with or without amifostine (200 mg/kg) or dexrazoxane (25 mg/kg) for 12 weeks

Treatment	No. of animals dead	Body weight (g) (mean ± SD)			
		0	6 doses	9 doses	12 doses
Doxorubicin	14/18	346 ± 18	345 ± 20*	324 ± 35*	316 ± 19*
Amifostine/doxorubicin	13/13	347 ± 20	337 ± 18*	259 ± 28*·**	–
Dexrazoxane/doxorubicin	2/18	340 ± 19	344 ± 9*	342 ± 16	346 ± 18*
Amifostine	0/5	328 ± 9	352 ± 13	380 ± 12	401 ± 7
Saline control	0/12	347 ± 22	377 ± 17	388 ± 23	396 ± 17

*$P < 0.05$, vs amifostine or saline control animals (Tukey-Kramer multiple comparisons test)
**$P < 0.05$, vs other groups

A significant increase in body weight was observed in the group of SHR receiving amifostine or saline without doxorubicin (Table 1). At the end of the study these animals had gained an average of 63 g and 49 g, respectively. Animals given doxorubicin alone had lost an average of 30 g body weight by the end of the study. The weights of the SHR in the group given doxorubicin in combination with dexrazoxane remained relatively constant and were significantly lower than those of the control groups ($P < 0.01$; Table 1). In contrast, SHR pretreated with amifostine lost an average of 88 g body weight after nine doses of doxorubicin. At that time, the average body weight in this group was significantly less than that in any other group ($P < 0.01$; Table 1).

Gross anatomic changes

At necropsy, the most consistent gross pathologic changes found in SHR given doxorubicin alone were excessive amounts of pericardial and peritoneal fluid and pale discoloration of the kidneys. Similar changes were found in the SHR treated with the combination of amifostine and doxorubicin. Animals treated with doxorubicin and dexrazoxane had less fluid accumulation than those receiving doxorubicin alone. No gross abnormalities were observed in the animals given amifostine or saline alone.

Myocardial alterations

Data on the incidence and severity of the myocardial lesions in the various groups of SHR are summarized in Table 2. These myocardial alterations (cytoplasmic vacuolization and loss of myofibrils) were identical to those previously found in SHR [19, 21], other animals [11, 17, 18], and patients [32] receiving doxorubicin. The SHR treated with doxorubicin alone had the most severe myocyte damage, with a majority (12 of 14) having cardiomyopathy scores of 2.5 or 3 (Table 2). Hearts from four other animals were not available for morphologic study. All 13 SHR given the combination of amifostine and doxorubicin died prior to termination of the study. Hearts from four animals were not available for evaluation. Cardiomyopathy scores ranged from 1 to 2.5 in the other nine animals in this group (Table 2). Eight of these animals had scores of 1 to 2, and the overall severity of the lesions in this group was significantly lower than that in the group receiving doxorubicin alone ($P < 0.01$). All animals given dexrazoxane and doxorubicin developed myocyte alterations (12 had cardiomyopathy scores of 1, 4 had scores of 1.5 and 1 had a score of 2), but these lesions were significantly less severe than those observed in SHR given doxorubicin alone or in combination with amifostine ($P < 0.05$; Table 2). No cardiac lesions were found in the hearts from the saline or amifostine control groups.

Pathology of noncardiac tissues

Nephrotoxicity was a consistent alteration in SHR given doxorubicin. The renal lesion scores were most severe (either 3+ in three animals or 4+ in nine animals) in SHR treated with doxorubicin alone (Table 3). Pretreatment with amifostine or dexrazoxane reduced the severity of the nephropathy to the extent that a majority of the SHR in these two groups had minimal (1+) (five animals in the amifostine group and four animals in the dexrazoxane group) or mild lesions (2+) (three animals in the amifostine group and ten animals in the dexrazoxane group; Table 3). The differences in the severity of the nephropathy scores in SHR given doxorubicin alone and those receiving the combinations of doxorubicin and either amifostine or dexrazoxane were highly significant ($P < 0.01$). However, there was no significant difference in nephropathy scores between the group receiving amifostine and doxorubicin and the group given dexrazoxane and doxorubicin. Samples of small intestine were obtained from eight SHR given doxorubicin. Morphological alterations consisting of minimal ($n = 2$) to marked ($n = 1$) epithelial cell loss and inflammation were noted in seven of these animals. Subtle or no change was found in the small intestine from SHR given amifostine or dexrazoxane prior to doxorubicin. No lesions were noted in the saline or amifostine control groups.

Discussion

The present study provides the first direct comparison of the protective effects of dexrazoxane and amifostine

Table 2 Cardiomyopathy scores in SHR given 12 weekly doses of doxorubicin (1 mg/kg) with or without amifostine (200 mg/kg) or dexrazoxane (25 mg/kg). The numbers of animals with each score are shown

Treatment	No. of hearts[a]	Cardiomyopathy score					
		0	1	1.5	2	2.5	3.0
Doxorubicin	14	0	0	2	2	7	3
Amifostine/doxorubicin**	9	0	3	2	3	1	0
Dexrazoxane/doxorubicin*,**	17	0	12	4	1	0	0
Amifostine	5	5	0	0	0	0	0
Saline control	12	12	0	0	0	0	0

*$P < 0.05$, vs scores of the group receiving amifostine and doxorubicin; **$P < 0.001$, vs scores of the group receiving doxorubicin alone; Mann-Whitney test

[a] The number of hearts available for analysis in each treatment group

Table 3 Nephropathy scores in SHR given 1 mg/kg doxorubicin weekly with or without amifostine (200 mg/kg) or dexrazoxane (25 mg/kg) for 12 consecutive weeks. The numbers of animals with each score are shown

Treatment	Number of kidneys[a]	Nephropathy score				
		0	1	2	3	4
Doxorubicin*	13	0	0	1	3	9
Amifostine/doxorubicin	10	0	5	3	2	0
Dexrazoxane/doxorubicin	18	1	4	10	3	0
Amifostine	5	5	0	0	0	0
Saline	11	11	1	0	0	0

*$P < 0.01$, vs scores of the groups receiving the combination of amifostine or dexrazoxane and doxorubicin; Mann-Whitney test

[a] Number of kidneys available for analysis in each treatment group

against doxorubicin toxicity. Both compounds attenuated the renal and cardiac toxicities, but only dexrazoxane protected against the loss of body weight and the lethality induced by doxorubicin.

Renal protection

The degree of renal and cardiac protection found in the present study is similar to that reported previously with dexrazoxane in SHR treated with doxorubicin [21]. Amifostine also has been found to protect against the nephrotoxicity induced by cisplatin in rats and mice [6, 39]. A similar protective activity has been observed in human clinical trials [15]. The mechanisms responsible for this protection are thought to involve hydrogen ion donation for DNA repair and scavenging of ROS [35].

Doxorubicin-induced renal and cardiac toxicity is thought to be mediated by the generation of ROS through redox cycling of the doxorubicin-Fe complex [2, 12, 13, 27]. Although this concept has not been completely validated, it is supported by considerable evidence [2, 12, 13, 16, 27]. However, other protective mechanisms also may be operative [25]. For reasons that are not clear, the renal toxicity of doxorubicin occurs only in rodents [38].

Cardiac protection

Dorr et al. [9] found that both amifostine and its metabolite, WR-1065, protected cultured neonatal rat cardiac myocytes against doxorubicin-induced loss of cell viability. This protective activity was apparent only when the myocytes were treated with these compounds prior to exposure to doxorubicin. Ohnishi et al. [28] have suggested that this cardioprotection was related to antioxidant activity, since both agents can reduce the ROS generated by doxorubicin in isolated rat heart mitochondrial preparations. Creatine kinase levels are significantly lower in tumor-bearing Balb/c mice that have received WR-1065 and doxorubicin than in those given doxorubicin alone [3]. Cardiac pathological changes induced by doxorubicin are reduced in mice treated with WR-1065. Nevertheless, it should be noted that the dose of doxorubicin employed in this study was considerably lower than that found by Bertazzoli et al. [1] to cause reproducible myocardial lesions in mice. Dobric et al. [7] have studied the efficacy of sodium selenite (1.6 mg/kg) and amifostine (300 mg/kg), singly and in combination, in reducing the cardiotoxicity produced by a single dose of 6 mg/kg doxorubicin in Wistar rats. The increase in the susceptibility to aconitine-induced arrhythmias and the rise in various serum enzyme levels following doxorubicin were best attenuated by pretreatment with both agents.

In the present study (Table 2), both amifostine and dexrazoxane attenuated doxorubicin-induced cardiotoxicity, but the cardiac protection was significantly greater, and the mortality was greatly decreased, in animals pretreated with dexrazoxane. Factors influencing this protection include: (1) cellular uptake, (2) dose and dosing frequency and (3) mechanism of action. Both dexrazoxane and amifostine are able to enter cells rapidly. Dexrazoxane given i.v. (100 mg/kg) to CDF-1 mice is concentrated maximally in heart, kidney and liver within 5 min [24]. This agent is taken up by isolated adult rat cardiac myocytes within 1 min of exposure to the drug [8]. Dexrazoxane is also rapidly converted to the open ring structure (ADR-925) [8, 16]. Amifostine given to Balb/c mice (500 mg/kg) is converted to the active free thiol metabolite (WR-1065) which accumulates in the heart and other tissues within 5–15 min [34]. Thus, it does not appear that differences in cellular uptake or conversion to an active metabolite are responsible for the more pronounced cardioprotection observed with dexrazoxane in the present study.

Previous cardioprotective studies with amifostine have utilized a pretreatment dosing schedule. However, the reported doses of this agent vary according to the cardiotoxicity model utilized. Bhanumathi et al. [3] reported that 50 mg/kg amifostine given daily over a 3-week period causes a decrease in the cardiotoxic effects of doxorubicin (0.85 mg/kg daily for 3 days/week for 3 weeks). A reduction in cardiotoxicity has also been found in rats given 300 mg/kg amifostine prior to 6 mg/kg doxorubicin [7]. Significant protection of noncardiac tissues has been observed in animals pretreated with 200 mg/kg amifostine, a dose that is considered to be [5] optimally radioprotective in rats.

Antioxidants such as *N*-acetylcysteine and vitamin E have been found to be ineffective in reducing chronic doxorubicin cardiotoxicity (summarized by Dorr [9]). Amifostine, an organic thiophosphate, is converted to

an active sulfhydryl metabolite (WR-1065) by membrane-bound alkaline phosphatases [34]. In vitro, WR-1065 has been found to directly detoxify both the superoxide anions and the hydroxyl radicals produced by doxorubicin [28]. Thus, ROS scavenging could be a mechanism by which amifostine causes a reduction in doxorubicin cardiotoxicity. Since other antioxidants have not modified this toxicity, it is possible that amifostine exerts other protective actions in addition to scavenging ROS [35]. In the present study, the doxorubicin-induced myocardial lesions in SHR pretreated with dexrazoxane were significantly less severe than those in animals pretreated with amifostine. Thus, it appears that an agent such as dexrazoxane, which prevents ROS generation, is more protective than one which scavenges ROS.

Protection against mortality and changes in body weight

The high mortality observed in the present study has not been reported in earlier studies of the use of amifostine against doxorubicin toxicity. The weekly administration of 1 mg/kg doxorubicin to SHR pretreated with amifostine caused gradual clinical deterioration, with death occurring at 9–11 weeks. Gastrointestinal lesions may have been a factor in the decline in body weight (10%) observed in SHR given doxorubicin alone over the 12-week course of the study. However, even more profound changes in body weight were observed in SHR pretreated with amifostine. The average body weight of these animals had declined over 20% from control levels after nine doses of doxorubicin and was significantly less than that observed in any other group of animals. This loss of body weight occurred even though the gastrointestinal toxicity was less than in SHR given doxorubicin alone. SHR given amifostine alone gained weight throughout the study. SHR pretreated with dexrazoxane did maintain their body weight in spite of doxorubicin treatment. Additional studies are needed to clarify the extent to which amifostine induces increased weight loss when combined with doxorubicin.

Schor [30] have observed that simultaneous administration of amifostine and 6-hydroxydopamine (an ROS generator) in mice results in enhanced toxicity (due to suppression of glutathione synthesis). Alterations in glutathione levels also may influence the toxic effects of doxorubicin.

Amifostine causes hypocalcemia in rats [22] and human patients [14, 37] due to inhibition of parathyroid hormone secretion and a decrease in tubular reabsorption of calcium. However, it was not possible to determine whether the combination of amifostine and doxorubicin caused more profound changes in serum calcium concentration. This was due to the fact that the mortality in this group of animals was 100% before the end of the study. Additional studies will be needed to evaluate this potential problem in detail.

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