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ORIGINAL ARTICLE

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Feasibility and pharmacokinetic study of infusional dexrazoxane and dose-intensive doxorubicin administered concurrently over 96 h for the treatment of advanced malignancies

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Abstract *Purpose*: Dexrazoxane administration prior to short infusion doxorubicin prevents anthracycline-related heart damage. Since delivery of doxorubicin by 96-h continuous intravenous infusion also reduces cardiac injury, we studied delivering dexrazoxane and doxorubicin concomitantly by prolonged intravenous infusion. *Methods*: Patients with advanced malignancies received tandem cycles of concurrent 96-h infusions of dexrazoxane 500 mg/m^2 and doxorubicin 165 mg/m^2, and 24 h after completion of chemotherapy, granulocyte-colony stimulating factor (5 μg/kg) and oral levofloxacin (500 mg) were administered daily until the white blood cell count reached 10,000 μl^{-1}. Plasma samples were analyzed for dexrazoxane and doxorubicin concentrations. *Results*: Ten patients were enrolled; eight patients had measurable disease. Two partial responses were observed in patients with soft-tissue sarcoma. The median number of days of granulocytopenia (<500 μl^{-1}) was nine and of platelet count $<20{,}000$ μl^{-1} was seven. Six patients received a single cycle because of progression (one), stable disease (four), or reversible, asymptomatic 10% decrease in cardiac ejection fraction (two). Principal grade 3/4 toxicities included hypotension (two), anorexia (four), stomatitis (four), typhlitis (two), and febrile neutropenia (seven), with documented infection (three). One death from neutropenic sepsis occurred. Dexrazoxane levels ranged from 1270 to 2800 n*M*, and doxorubicin levels ranged from 59.1 to 106.9 n*M*. *Conclusions*: These results suggest that tandem cycles of concurrent 96-h infusions of dexrazoxane and high-dose doxorubicin can be administered with minimal cardiac toxicity, and have activity in patients with recurrent sarcomas. However, significant non-cardiac toxicities indicate that the cardiac sparing potential of this approach would be maximized at lower dose levels of doxorubicin.

Keywords Pharmacokinetcs · Anthracycline · Cardiotoxicity · Dexrazoxane · Doxorubicin

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Introduction

The development of congestive heart failure (CHF) is a serious sequela of anthracycline use. Known factors that enhance the risk of developing doxorubicin-related heart damage include cumulative anthracycline use, cardiac irradiation, age, and preexisting cardiac disease [3, 4, 24, 38]. A retrospective analysis of three phase III studies of patients who were randomized to a doxorubicin plus placebo arm showed a 5.1% incidence of doxorubicin-related CHF [34]. Furthermore, up to 5% of children who receive an anthracycline for treatment of childhood cancer have been observed to develop clinical heart failure after 15 years [19]. Therefore, strategies to minimize this toxicity have been aggressively pursued. Dexrazoxane is a bidentate chelator of divalent cations that, after hydrolysis, resembles EDTA. Dexrazoxane has been shown to reduce the cardiotoxicity of anthracyclines such as doxorubicin in preclinical studies [14–16]. Dexrazoxane does not inhibit the antitumor

effect of doxorubicin [40]. In contrast, dexrazoxane and doxorubicin synergistically inhibit the proliferation of murine sarcoma cells in vitro [40]. The cardioprotective effect of dexrazoxane is, at least in part, related to its chelation of unbound transition metals, and subsequent inhibition of free radical generation. Clinically, dexrazoxane has been shown to reduce the incidence and severity of cardiomyopathy in women with metastatic breast cancer receiving bolus doxorubicin in several trials [30–33].

The cellular pharmacology of dexrazoxane has been examined in beating heart myocytes of adult rats [7]. Drug uptake of radiolabeled dexrazoxane is rapid, with maximum levels of myocyte-associated radioactivity detected within 60 s of drug exposure. Efflux of myocyte-associated dexrazoxane and its hydrolysis products are equally rapid, and essentially complete within 1 min. The uptake and efflux of the drug are energy-independent and temperature-independent, and thus, are likely to be diffusion-mediated. These results suggest that the clinical application of dexrazoxane would be amenable to continuous intravenous (i.v.) infusion.

Antitumor activity of ICRF-159, the racemic form of dexrazoxane, has been demonstrated clinically in non-small-cell lung cancer [8], colorectal carcinoma [2, 22], Kaposi's sarcoma [26], non-Hodgkin's lymphoma [12], acute leukemia [1], and head and neck carcinoma [27]. A phase I trial of fixed dose doxorubicin (60 mg/m^2) preceded by dexrazoxane in escalating doses has been reported [18]. The dexrazoxane was administered over a 15-min period beginning 30 min before doxorubicin treatment; the protocol was repeated every 3 weeks. Dose-limiting neutropenia occurred in four of six previously treated patients with dexrazoxane 600 mg/m^2. Grade 3/4 neutropenia occurred in four of six patients treated with a maximum of one prior chemotherapy regimen who received dexrazoxane at a dose of 900 mg/m^2 and in three of four patients at a dose of 750 mg/m^2. Pharmacokinetic analyses failed to demonstrate alteration in the distribution, metabolism, or excretion of doxorubicin.

On the basis of the rapid, diffusion-mediated cellular uptake and short plasma half-life of dexrazoxane, we designed a phase I pharmacokinetic trial of a 96-h continuous infusion of dexrazoxane with granulocyte-colony stimulating factor support [37]. Dose-limiting toxicities were limited to mild thrombocytopenia, diarrhea, hypertension, and nausea and vomiting. At doses ranging from 125 to 250 mg/m^2 per day, steady-state dexrazoxane levels ranged from 496 μg/l (2.2 μ*M*) to 1639 μg/l (7.4 μ*M*). Although these levels were above the levels required to induce cytotoxicity in K562 cells, an erythroleukemic cell line (IC_{50} 3.6 μ*M* [36]), no objective responses were observed in the 21 evaluable patients.

Doxorubicin cardiotoxicity is decreased by prolonged continuous i.v. infusion without compromising its antitumor activity [6, 20, 35], in part by diminishing its peak plasma level. Because prior clinical trials with dexrazoxane as an anthracycline cardioprotectant had utilized doxorubicin by i.v. bolus [18, 30–33], and based upon our phase I trial of dexrazoxane delivered by 96-h continuous i.v. infusion, we designed a pilot feasibility trial of tandem cycles of 96-h continuous i.v. infusions of the combination of dexrazoxane and dose-intensive doxorubicin for the treatment of advanced malignancies or malignancies considered at high risk of recurrence. We hypothesized that increasing doxorubicin dose-intensity might improve response rate. Moreover, we theorized that the cardioprotectant effect of a continuous infusion of dexrazoxane, if delivered in the appropriate molar ratio vs the anthracycline, would be maintained in spite of the dose intensity of the doxorubicin.

Materials and methods

Study design

This study was a single-center pilot trial designed to study the feasibility and pharmacokinetics of delivering concurrent dexrazoxane and high-dose doxorubicin by 96-h continuous i.v. infusion, and to compare the pharmacokinetics of the two drugs when administered in combination with the pharmacokinetics of 96-h continuous i.v. infusion of doxorubicin or dexrazoxane each delivered alone in previous trials [35, 37]. The planned enrollment was ten patients. This permitted a detection of a 16.4% change in the geometric mean area under the curve (AUC) with 80% power, using a two-sided, 0.05-level test compared to historical pharmacokinetic data from 20 patients given doxorubicin or dexrazoxane alone.

Patient eligibility

All patients entered into this study were at least 18 years of age, had either histologically proven advanced cancer, or were selected for having the potential to benefit from high-dose doxorubicin. Patients were required to possess a Karnofsky performance status of ≥70%. Patients may have received previous doxorubicin but must not have received a cumulative dose of more than 300 mg/m^2. Patients were required to have adequate bone marrow function defined as a neutrophil count of at least 2000 μl^{-1}, a platelet count of at least 120,000 μl^{-1}, and adequate renal function defined as a creatinine clearance ≥50 ml/min. Bilirubin was required to be no greater than 1.5 mg/dl, and aspartate aminotransferase and alanine aminotransferase less than 2.5 times the upper limit of normal. Patients were required to have a left ventricular ejection fraction by multiple-gated blood pool (MUGA) scan of $>50\%$. Prior radiation to ≥20% of the bone marrow was an exclusion criterion, as was prior nitrosourea chemotherapy. All patients provided informed, voluntary consent, and signed an informed consent document approved by the Cancer Protocol Review and Monitoring Committee, and Institutional Review Board at the City of Hope National Medical Center.

Pretreatment evaluation

Pretreatment evaluation included a complete history and physical examination, complete blood count with differential, a chemistry panel that included liver function tests and serum creatinine, serum magnesium level, chest radiograph, MUGA scan, electrocardiogram, urinalysis, and a 24-h urine collection for creatinine clearance. Patients with bidimensionally measurable disease were required to have baseline evaluations within 4 weeks before the first course of therapy. Radiologic evaluations for repeat tumor assessments and MUGA scans were planned after each course of therapy.

Treatment plan

Dexrazoxane 500 mg/m^2 and doxorubicin 165 mg/m^2 were administered as 96-h continuous i.v. infusions to inpatients. Dexrazoxane was shown to be stable in 5% dextrose diluted to either 0.1 or 0.5 mg/ml with $>90\%$ of its initial concentration found at up to 24 h [37]. Therefore, all patients received four consecutive 24-h infusions of dexrazoxane diluted to ≤ 0.5 mg/ml in 5% dextrose along with the doxorubicin infusion. This dose of dexrazoxane (125 mg/m^2 per day) was chosen based upon our prior phase I trial that demonstrated a steady-state dexrazoxane concentration of about 2 μM at this drug level, an approximately 20-fold molar excess over the doxorubicin levels of about 0.1 μM documented by our prior investigations utilizing a 96-h continuous infusion of doxorubicin at 165 mg/m^2 [35, 37]. This more than tenfold excess of dexrazoxane to doxorubicin has been shown to maximize the cardioprotection provided by the bis-dioxopiperazine [7, 16]. Filgrastim was administered at a dose of 5 μg/kg as a single daily subcutaneous injection beginning 24 h after completion of chemotherapy until the white blood cell count exceeded 10,000 μl^{-1}. Daily oral levofloxacin was administered on the same days as the filgrastim injection. A second course of therapy was administered within 6 weeks of the start of the first course of treatment in the absence of unacceptable toxicity or tumor progression.

Pharmacokinetic studies

For determination of dexrazoxane and doxorubicin plasma pharmacokinetics, 20 ml of peripheral blood was obtained in vacuum tubes containing sodium heparin during the first course of therapy at the following times: prior to the start of the infusion; at 24, 48, 72 and 96 h during the infusion; and then at 24 and 48 h after the end of the infusion. Samples were centrifuged at 1500 *g* for 10 min, and the plasma was separated; 50 μl of phosphoric acid (42.5% v/v) was added to each 1 ml of plasma to prevent ex vivo hydrolysis of dexrazoxane. Plasma was stored at −70°C until analysis by high-performance liquid chromatography (HPLC).

The HPLC method of detection of dexrazoxane levels consisted of gradient separation across a C_{18} column (150×4.6 mm; Phenomenex, Torrance, Calif.) and UV detection at a wavelength of 209 nm. Mobile phase A was 0.01 *M* phosphate buffer (pH 4.7) with 0.1 m *M* EDTA, and mobile phase B was 100% methanol. The gradient program was as follows: linear increase from 2% to 7% B by 8 min, hold at 7% B until 18 min; linear increase from 7% to 15% by 22 min, hold at 15% B until 31 min; linear decrease from 15% to 2% B by 33 min; and equilibrate at 2% B until 40 min. The flow rate was constant at 1.2 ml/min. Using the HPLC conditions above, the retention times for dexrazoxane and the internal standard were 15.4 and 28.8 min, respectively. The mean percentage recovery was 101.1% across the entire range of the standard curve. Interday and intraday precision and accuracy were within 10% of the target value. The lower limit of detection was 10 ng/ml, and the lower limit of quantitation, defined as a peak height/baseline noise ratio of ≥3, was 20 ng/ml.

Plasma doxorubicin concentrations were determined using a previously described loop-column method [35]. Briefly, with a manual injector turned to the load position, plasma was injected directly onto a guard column containing a phenyl packing material. The guard column was washed with deionized water, and the injector turned to the inject position, allowing the mobile phase to carry the sample through the HPLC system. Separation was achieved with elution across a phenyl analytical column, and detection carried out by fluorescence using a λ_{ex} of 507 n *M* and λ_{em} of 550 n*M*. Daunorubicin was added to each plasma sample prior to injection as an internal standard.

Pharmacokinetic data analyses of individual plasma drug concentration vs time curves were performed using ADAPT II software (Biomedical Simulations Resource, University of Southern California, Los Angeles, Calif.). Both doxorubicin and dexrazoxane concentrations were fitted to a two-compartment pharmacokinetic model with first-order elimination. Adequacy of the model-derived fits was assessed by the examination of residuals between predicted and measured drug concentrations, along with the correlation coefficients. Doxorubicin and dexrazoxane systemic clearances (Cl_{sys}) were determined using the model-derived pharmacokinetic parameters, while the doxorubicin end of infusion concentrations (EOI) and dexrazoxane steady-state levels were defined as the actual measured values. The difference in measured values and those simulated using the individual model-derived parameters were in very good agreement for all patients.

Evaluation of toxicity and efficacy

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (CTC), version 2.0, using the bone marrow transplantation criteria of CTC v2.0 for hematologic toxicities. World

Health Organization response criteria were used to evaluate for objective responses in patients with bidimensionally measurable lesions [23]. Responses were confirmed by a second evaluation ≥1 month after the evaluation documenting response. Response duration was defined as time from first objective status assessment of response to the first time of progression or death due to any cause.

Results

Patient characteristics

A total of ten patients were enrolled in this study. All ten patients were fully assessable for toxicity, and eight were assessable for response. Two patients were treated adjuvantly, one each with soft-tissue sarcoma and thymoma. Of the ten patients entered in this study, there were six with soft-tissue sarcomas (60%), and one each with thymoma, ovarian cancer, breast cancer, and adenoid cystic carcinoma of the salivary gland. The demographic features of the patients are shown in Table 1. All ten patients had prior surgical resection, and nine had received prior chemotherapy before study entry. The patient with thymoma had received prior adjuvant chemotherapy with cyclophosphamide, doxorubicin, vincristine, and cisplatin (CHOP), and etoposide, ifosfamide, and cisplatin (VIP) for recurrence. The median number of prior chemotherapy treatments was one (range zero to three).

Toxicity

A total of 14 treatment courses were administered. Four patients received the full prescribed course of two cycles of treatment; six patients received only a single cycle of treatment. Among the six patients receiving one course of treatment, one patient received treatment adjuvantly and refused the second course of treatment (thymoma), four patients had stable disease and declined the second course of treatment (neurofibrosarcoma, ovarian, breast, and adenoid cystic carcinomas), and one patient had progressive disease (fibrosarcoma). All toxicities for which there was greater than one drug-related occurrence graded 3 or higher on either course of treatment are shown in Table 2. As expected, significant but reversible hematologic toxicity was observed. Grade 4 neutropenia developed after every treatment course ($< 100\ \mu l^{-1}$). The median duration of absolute neutropenia $< 500\ \mu l^{-1}$ was 9 days (range 5–33 days). Fever occurred in 12 of the treatment courses (ten patients); in three of the courses, a documented source of infection

Table 2 Grades 3, 4, or 5 toxicities for all courses of treatment ($N = 14$)

Toxicity	Grade 3	Grade 4	Grade 5
Electrolytes			
Dehydration	2		
Hypocalcemia		2	
Hypokalemia	2	1	
Hypophosphatemia	1	1	
Gastrointestinal			
Anorexia	4	1	
Dysphagia/stomatitis	5	1	
Nausea	2		
Hematologic			
Anemia	6	1	
Hemorrhage	3		
Leukocytes		14	
Neutrophils	1	13	
Thrombocytopenia	3	8	
Prothrombin time	2		
Infections			
Febrile neutropenia	7		
Infection	2	1	
Typhlitis	1		1
Pulmonary			
Dyspnea	2	1	
Hypoxia	1	1	
Other			
Hallucinations	3		
Hypotension	1	1	
Syncope	2		

Table 1 Patient characteristics

Histology	No. of patients	Gender (M/F)	Age (years)	Karnofsky performance status (%)	No. of prior chemotherapy regimens	Prior doxorubicin (prior cumulative dose)
Thymoma	1	F	44	90	2	Yes (200 mg/m^2)
Ovarian cancer	1	F	48	90	1	No
Breast cancer	1	F	57	100	3	No
Adenoid cystic carcinoma	1	F	53	70	1	No
Sarcoma	6					
Rhabdomyosarcoma	1	M	34	90	3	No
Carcinosarcoma	1	F	53	80	1	Yes (300 mg/m^2)
Liposarcoma	1	M	39	90	1	Yes (225 mg/m^2)
Fibrosarcoma	1	M	44	80	3	Yes (270 mg/m^2)
Epithelioid sarcoma	1	F	32	90	0	No
Neurofibrosarcoma	1	M	26	80	2	Yes (120 mg/m^2)

was identified (one patient each with: *Candida albicans* and herpes simplex pneumonia, coagulase-negative *Staphylococcus* intravascular catheter sepsis, and *Clostridium difficile* gastroenteritis/typhlitis). A patient with neutropenic typhlitis died as a result of septic complications after the second cycle. Grade 3/4 thrombocytopenia developed after every treatment course. The median duration of thrombocytopenia $<20{,}000\ \mu l^{-1}$ was 7 days (range 1–11 days). Other significant, nonhematologic toxicities included: anorexia, dyspnea, dysphagia/stomatitis, bleeding, hypoxia, nausea, and typhlitis.

There was no clinically significant cardiac toxicity noted. An asymptomatic, grade 1 decline in left ventricular ejection function (LVEF) was observed in two patients after a single course of treatment. The first patient had a 10% absolute decline in LVEF (60% to 50%). She had received prior mediastinal radiation for thymoma (4320 cGy by external beam and 1200 cGy by intraoperative radiation), and 200 mg/m^2 of doxorubicin given by bolus injection. Her LVEF returned to baseline when measured 28 months later (59%). The second patient had an 11% decline in LVEF (74% to 63%). She had a history of poorly controlled hypertension. She had not received prior doxorubicin before undergoing protocol treatment. Further follow-up of LVEF was not performed. A second course of treatment was not offered to these patients due to their grade 1 decline in LVEF.

Responses

Among the eight patients assessable for response, two achieved an objective partial response. The first patient had recurrent alveolar rhabdomyosarcoma. He was diagnosed at age 32 years with stage T3N1M0 disease in the right neck with right axillary adenopathy, and was treated with five courses of vincristine, actinomycin-D, and cyclophosphamide, followed by radiation, achieving a complete remission. He relapsed locally within 8 months, and progressed despite first-line and second-line salvage chemotherapy (ifosfamide and etoposide, high-dose ifosfamide). He achieved a partial remission after the first cycle of protocol treatment, which was maintained until 4 months after completion of the second cycle of protocol treatment. He eventually died of progressive disease. A second patient with radiation-induced carcinosarcoma of the nasopharynx achieved a sustained partial remission. She developed gastric adenocarcinoma at age 43 years. She received postoperative, adjuvant 5-fluorouracil, doxorubicin, vincristine, and mitomycin-C. The cumulative dose of doxorubicin received was 300 mg/m^2. She developed a solitary brain metastasis less than 1 year later, which was surgically resected and treated with external beam radiation. At age 53 years, 10 years later, she developed a radiation-induced nasal cavity carcinosarcoma, which was surgically removed. She developed a large, local recurrence within 6 months of surgery, which was no longer amenable to surgery or radiation. She achieved a partial remission after the first cycle of protocol treatment. Continued response was noted after the second cycle of protocol treatment. She received six additional cycles of carboplatin and etoposide due to the presence of small volume residual mass, although there was no change in the size of the mass during and after completion of carboplatin and etoposide. At the time of this report, there was no evidence of tumor regrowth at 53 months after completion of protocol therapy.

Pharmacokinetics

The results of the dexrazoxane and doxorubicin plasma pharmacokinetic analyses are shown in Table 3 and Figs. 1 and 2. The mean steady-state dexrazoxane level was 1974 n *M* (range 1270–2800 n *M*), and the mean end-of-infusion doxorubicin level was 83.8 n *M* (range 59.1–106.9 n *M*). Dexrazoxane and doxorubicin Cl_{ss} were 12.6 ± 3.1 l/h/m^2 (range 8.4–18.6 l/h/m^2) and 23.5 ± 3.4 l/h/m^2 (range 19.3–29.5 l/h/m^2), respectively.

Table 3 Dexrazoxane and doxorubicin plasma pharmacokinetics (*EOI* end of infusion concentration, Cl_{sys} systemic clearance, C_{ss} steady-state concentration)

Patient	Doxorubicin		Dexrazoxane		Dex/Dox
	EOI concentration (nM)	Cl_{sys} (l/h/m^2)	C_{ss} (nM)	Cl_{sys} (l/h/m^2)	
1	76.9	20.5	2610	9.0	33.9
2	78.0	25.0	1550	15.2	19.9
3	94.9	25.2	1910	12.4	20.1
4	94.9	20.8	2000	11.8	21.1
5	98.2	29.5	1930	12.2	19.6
6	59.1	20.5	1270	18.6	21.5
7	76.2	21.9	1600	14.8	21.0
8	79.5	25.0	2310	10.2	29.1
9	74.0	27.8	1760	13.4	23.8
10	106.9	19.3	2800	8.4	26.2
Average	83.8	23.5	1974	12.6	23.6
SD	14.4	3.4	479	3.1	4.7

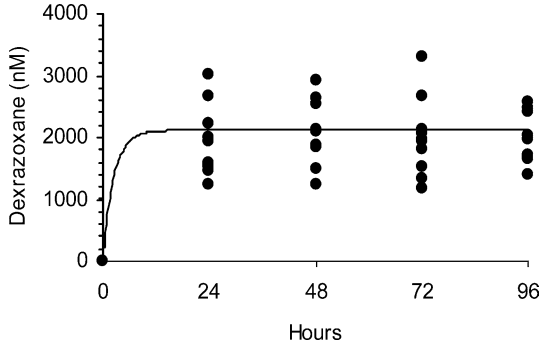

Fig. 1 Steady-state concentration of dexrazoxane. The figure demonstrates the steady-state concentration of dexrazoxane. A mean steady-state concentration of 1974 n*M* (range 1270–2800 n*M*, SD 479 n*M*) was achievable by 24 h

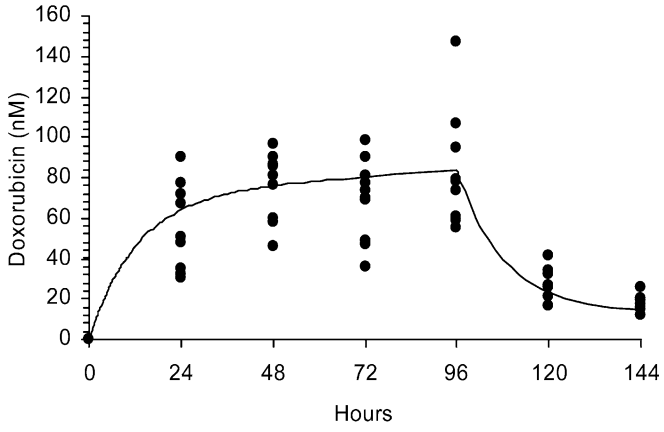

Fig. 2 Plasma concentrations of doxorubicin. This figure demonstrates the plasma concentrations of doxorubicin at the indicated time points sampled. The mean end-of-infusion doxorubicin concentration was 83.8 n*M* (range 59.1–106.9 n*M*, SD 14.4 n*M*). The plasma concentration of doxorubicin rapidly declined upon completion of the doxorubicin infusion

Plasma dexrazoxane levels exceeded plasma doxorubicin levels by a mean of 23.6-fold (range 19.9–33.9).

Discussion

Doxorubicin has a major role in the treatment of numerous solid tumors and hematologic malignancies in both adult and pediatric patients. It has improved event-free survival for women with early breast cancer and for many pediatric cancers [10, 11, 17, 28, 41], and its palliative role in women with advanced breast cancer is clearly established [9]. Despite this, its use is constrained by its most important clinical side effect, cumulative and dose-dependent cardiotoxicity. The dose-dependency of doxorubicin-induced cardiotoxicity was illustrated more than two decades ago by Von Hoff et al. in which the cumulative probability of developing drug-induced CHF was 0.03 at 400 mg/m^2, 0.07 at 550 mg/m^2, and 0.18 at 700 mg/m^2 in both children and adults. The probability of CHF increased dramatically after a total dose of 550 mg/m^2 was reached [38].

Previous studies have clearly demonstrated that cardiotoxicity induced by short infusions of doxorubicin may be reduced by pretreatment with dexrazoxane [18, 30–33]. The optimal dexrazoxane/doxorubicin dose ratio in these studies was determined to be 10:1 [32, 33]. Additionally, delayed administration of dexrazoxane provided significant cardioprotection for patients with advanced breast cancer even after patients had reached a cumulative doxorubicin dose of 300 mg/m^2 [32].

We have been interested in dose-intensive, prolonged infusion doxorubicin as a means of overcoming intrinsic chemotherapy resistance while minimizing cardiac toxicity [25, 29]. Previously, we reported a trial of tandem cycles of dose-intensive doxorubicin (150 mg/m^2) and cyclophosphamide (4.2 g/m^2) in patients with high-risk or metastatic breast cancer [25]. Pretreatment and post-treatment endomyocardial biopsies documented that no significant cardiac damage occurred. However, patients entered on these trials had received ≤ 180 mg/m^2 of doxorubicin previously. To pursue this approach further and increase cardiac protection so as to expand the range of patients eligible for such treatment, we were interested in the use of dexrazoxane by infusion as a cardioprotectant in combination with prolonged infusions of doxorubicin. In our preliminary phase I trial of 96-h prolonged infusion dexrazoxane followed by G-CSF support, we established the maximal tolerated dose (MTD) of dexrazoxane to be 665 mg/m^2/96 h [37]. The dose-limiting toxicities were thrombocytopenia, gastrointestinal toxicity, and hypertension; whereas, in earlier phase I studies of dexrazoxane given as a short infusion, myelosuppression (particularly thrombocytopenia) was the major toxicity [21, 39]. This demonstrates the schedule-dependent toxicity of this drug. The results of our phase I study encouraged us to examine whether giving both agents as concurrent 96-h infusions was feasible.

The MTD (on a milligram basis) of dexrazoxane for our phase I trial (665 mg/m^2) was four times the dose of doxorubicin used in our dose-intensive doxorubicin trials (165 mg/m^2). Our pharmacokinetic studies demonstrated that the steady-state plasma levels of dexrazoxane at the MTD were about 18 times greater than those of doxorubicin [35, 37]. Accordingly, we designed the current trial to simultaneously combine 96-h continuous infusions of dexrazoxane 500 mg/m^2 and doxorubicin 165 mg/m^2. As we anticipated, the pharmacokinetic studies from the present trial confirmed that the plasma levels of dexrazoxane exceeded the plasma doxorubicin levels by a mean of 24-fold, and steady-state levels of both dexrazoxane and doxorubicin were achieved by 24 h. Whereas the hematologic toxicity of infusional dexrazoxane and dose-intensive doxorubicin is severe, but manageable with blood product and growth factor support, cardiac toxicity is minimal. There was no clinically significant cardiac toxicity noted in this study. Of the two patients with ≥10% asymptomatic decline in LVEF after one course of therapy, the LVEF of one patient had returned to baseline when measured more than 2 years later. These results support our original hypothesis that simultaneous administration of 96-h continuous infusions of dexrazoxane and high-dose doxorubicin can be accomplished with minimal cardiotoxicity.

We previously reported the effect of dose on the pharmacokinetics of doxorubicin administered as a 96-h continuous infusion [35]. In that study, we compared previously published pharmacokinetic data: 31.9 n*M* for 60 mg/m^2, 47.1 n*M* for 100 mg/m^2 to our pharmacokinetic data for doxorubicin 165 mg/m^2 (102.2 ± 7.2 n*M*) [5]. Doxorubicin plasma levels were dose-dependent, strongly suggesting linear pharmacokinetic behavior. Results from the present trial confirm our previous findings. When combined with infusional dexrazoxane, the mean end-of-infusion doxorubicin level and systemic clearance are well within the confidence intervals of our

prior study. Consequently, the addition of concurrent continuous infusion dexrazoxane does not appear to significantly alter the pharmacokinetics of 96-h continuous infusion doxorubicin.

In contrast, the pharmacokinetics of dexrazoxane appear to be significantly affected by the concurrent administration of doxorubicin. We previously determined the clearance of single-agent dexrazoxane administered as a 96-h continuous to be 6.7 ± 1.6 l/h/m^2 [37]. However, in the present trial, we found that the systemic clearance of dexrazoxane was approximately twice as fast when combined with infusional doxorubicin (12.6 ± 3.1 l/h/ m^2). The increased clearance of dexrazoxane in the presence of doxorubicin was reflected in the mean steady-state concentration of dexrazoxane determined in the two trials. In the current study, the mean steady-state concentration (1974 ± 479 n *M*) was twofold lower than the mean level seen with single-agent dexrazoxane given at the same dose (4006 ± 806 n *M*) [37].

One possible explanation for the increased clearance and resulting lower steady-state dexrazoxane concentrations may be an interaction between circulating doxorubicin-metal complexes in plasma and dexrazoxane [13]. Hasinoff et al. have demonstrated that both Fe^{3+}-doxorubicin and Cu^{2+}-doxorubicin react directly with dexrazoxane promoting a ring-opening hydrolysis of dexrazoxane that results in displacement of the metal ion from its complex with doxorubicin. Evidence in support of this metal ion complex-promoted hydrolysis includes the identification of a mixed ligand complex, which exhibits saturation behavior at high dexrazoxane concentrations. Further, direct spectroscopic evidence of both a Cu^{2+}–doxorubicin–dexrazoxane mixed ligand complex and a Cu^{2+}–(doxorubicin–dexrazoxane)2 complex is demonstrable [13]. We believe these in vitro results are consistent with our in vivo finding that dexrazoxane clearance may be enhanced through hydrolysis in the setting of a concurrent doxorubicin infusion. To the extent that this hypothesis has merit, the presence of circulating doxorubicin/metal complexes in vivo provides additional support for the possible need for a continuous presence of the cardioprotective agent.

Although high-dose doxorubicin-induced cardiac toxicity can be prevented by the simultaneous prolonged infusion of dexrazoxane, hematologic toxicity proved limiting in this trial. Despite the use of prophylactic filgrastim and prophylactic broad-spectrum oral antibiotic, grade 4 neutropenia and thrombocytopenia developed after every treatment course, and febrile neutropenia or neutropenia with infection occurred in 12 of the 14 treatment courses. A grade 5 neutropenic typhlitis was also observed. As a result, the present regimen at these doses cannot be recommended outside a clinical trial.

Despite the hematologic toxicity, gratifying responses were observed in this trial. Of the eight patients with advanced disease, two partial remissions were observed among patients with recurrent soft-tissue sarcoma who had received prior chemotherapy. The current trial represented third-line salvage chemotherapy of chemotherapy-refractory disease for the patient with recurrent rhabdomyosarcoma. For the patient with radiation-induced carcinosarcoma, the current trial represented first-line chemotherapy, although 10 years earlier she had received a cumulative dose of 300 mg/m^2 doxorubicin for adjuvant therapy of gastric cancer. At the time of writing, she remained in sustained partial remission 53 months after participation in this clinical trial. These results suggest that concurrent prolonged administration of dexrazoxane and high-dose doxorubicin has significant activity in the therapy of recurrent soft-tissue sarcomas. As a result, we have opened a phase II trial of this regimen for recurrent soft-tissue and bone sarcomas. To reduce hematologic toxicity in the phase II setting, we have reduced the dose of doxorubicin and dexrazoxane in the second course to 120 and 365 mg/m^2, respectively.

In summary, this pilot feasibility study represents the first reported clinical trial of concurrent administration of a 96-h continuous infusion of dexrazoxane and high-dose doxorubicin. As more pediatric clinical trials employ dose-intensive strategies with prolonged doxorubicin infusions and higher cumulative doses, the present approach may offer a strategy to reduce the risk of developing late doxorubicin-associated cardiomyopathy.

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References

1. Bakowski MT, Prentice HG, Lister TA, Malpas JS, McElwain TJ, Powles RL (1979) Limited activity of ICRF-159 in advanced acute leukemia. Cancer Treat Rep 63:127
2. Bellet RE, Engstrom PF, Catalano RB, Creech RH, Mastrangelo MJ (1976) Phase II study of ICRF-159 in patients with metastatic colorectal carcinoma previously exposed to systemic chemotherapy. Cancer Treat Rep 60:1935
3. Billingham ME, Bristow MR, Glatstein E, Mason JW, Masek MA, Daniels JR (1975) Adriamycin cardiotoxicity: endomyocardial biopsy evidence of enhancement by irradiation. Am J Surg Pathol 1:17
4. Bristow MR, Thompson PD, Martin RP, Mason JW, Billingham ME, Harrison DC (1978) Early anthracycline cardiotoxicity. Am J Med 65:823
5. Bugat R, Robert J, Herrera A, Pinel MC, Huet S, Chevreau C, Boussin G, Roquain J, Carton M (1989) Clinical and pharmacokinetic study of 96-h infusions of doxorubicin in advanced cancer patients. Eur J Cancer Clin Oncol 25:505
6. Casper ES, Gaynor JJ, Hajdu SI, Magill GB, Tan C, Freidrich C, Brennan MF (1991) A prospective randomized trial of adjuvant chemotherapy with bolus versus continuous infusion of doxorubicin in patients with high-grade extremity soft tissue sarcoma and an analysis of prognostic factors. Cancer 68:1221
7. Doroshow JH (1995) Role of reactive-oxygen metabolism in the cardiac toxicity of the anthracycline antibiotics. In: Priebe W (ed) Anthracycline antibiotics: new analogues, methods of delivery, and mechanisms of action. American Chemical Society, Washington, p 259
8. Eagan RT, Carr DT, Coles DT, Rubin J, Frytak S (1976) ICRF-159 versus polychemotherapy in non-small cell lung cancer. Cancer Treat Rep 60:947

9. Ellis MJ, Hayes DF, Lippman ME (2000) Treatment of metastatic breast cancer. In: Harris JR, Lippman ME, Morrow M (eds) Diseases of the breast (2nd edn). Lippincott Williams & Wilkins, Philadelphia, p 749
10. Fisher B, Redmond C, Wickerham KL, Bowman D, Schipper H, Wolmark N, Sass R, Fischer ER, Jochimsen P, Legault-Poisson S (1989) Doxorubicin-containing regimens for the treatment of stage II breast cancer: the National Surgical Adjuvant Breast and Bowel Project experience. J Clin Oncol 7:572
11. Fisher B, Redmonds C, Legault-Poisson S, Dimitrov NV, Brown AM, Wickerham KL, Wolmark N, Margolese RG, Bowman D, Glass AG (1990) Post-operative chemotherapy and tamoxifen compared with tamoxifen alone in the treatment of positive-node breast cancer patients aged 50 years and older with tumors responsive to tamoxifen: results from the National Surgical Adjuvant Breast and Bowel Project B-16. J Clin Oncol 8:1005
12. Flannery EP, Corder MP, Shehan WW, Pajak TF, Bateman JR (1978) Phase II study of ICRF-159 in non-Hodgkin's lymphomas. Cancer Treat Rep 62:465
13. Hasinoff BB (1990) The iron(III) and copper(II) complexes of adriamycin promote the hydrolysis of the cardioprotective agent ICRF-187 ((+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane). Agents Actions 29:374
14. Herman EH, Mhatre RM, Lee IP, Waravdekar VS (1972) Prevention of the cardiotoxic effects of adriamycin and daunomycin in the isolated dog heart. Proc Soc Exp Biol Med 140:234
15. Herman EH, Mhatre RM, Chadwick DP (1974) Modification of some of the toxic effects of daunomycin (NSC-82, 151) by treatment with the antineoplastic agent ICRF-159 (NSC-129,943). Toxicol Appl Pharmacol 27:517
16. Herman EH, Ferrans VJ, Jordan W, Ardalan B (1981) Reduction of chronic daunorubicin cardiotoxicity by ICRF-187 in rabbits. Res Commun Chem Pathol Pharmacol 31:85
17. Hitchcock-Bryan S, Gelber R, Cassady JR, Sallan SE (1986) The impact of induction anthracycline on long-term failure-free survival in childhood acute lymphoblastic leukemia. Med Pediatr Oncol 14:211
18. Hochster H, Liebes L, Wadler S, Oratz R, Wernz JC, Meyers M, Green M, Blum RH, Speyer JL (1992) Pharmacokinetics of the cardioprotector ADR-529 (ICRF-187) in escalating doses combined with fixed-dose doxorubicin. J Natl Cancer Inst 84:1725
19. Kremer LCM, van Dalen EC, Offringa M, Ottenkamp J, Voûte PA (2001) Anthracycline-induced clinical heart failure in a cohort of 607 children: long-term follow-up study. J Clin Oncol 19:191
20. Legha SS, Benjamin RS, Mackay B, Ewer M, Wallace S, Valdivieso M, Rasmussen SL, Blumenschein GR, Freireich EJ (1982) Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 96:133
21. Liesmann J, Belt R, Haas C, Hoogstraten B (1981) Phase I evaluation of ICRF-187 (NSC-169780) in patients with advanced malignancy. Cancer 47:1959
22. Marciniak TA, Moertel CG, Schutt AJ, Hahn RG, Reitemeir RJ (1975) Phase II study of ICRF-159 (NSC-129943) in advanced colorectal carcinoma. Cancer Chemother Rep 59:761
23. Miller AB, Hoogstraten B, Staquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47:207
24. Minow RA, Benjamin RS, Gottlieb JA (1975) Adriamycin (NSC-123127) cardiomyopathy—an overview with determination of risk factors. Part 3. Cancer Chemother Rep 6:195
25. Morgan RJ Jr, Doroshow JH, Venkataraman K, Chang K, Raschko J, Somlo G, Leong L, Tetef M, Shibata S, Hamasaki V, Margolin K, Forman S, Akman S, Coluzzi P, Ahn C, Weiss L, Gadgil U, Harrison J (1997) High-dose infusional doxorubicin and cyclophosphamide: a feasibility study of tandem high-dose chemotherapy cycles without stem cell support. Clin Cancer Res 3:2337
26. Olweny CL, Masaba JP, Sikyewunda W, Toya T (1976) Treatment of Kaposi's sarcoma with ICRF-159 (NSC 129943). Cancer Treat Rep 60:111
27. Shah MK, Engstrom PF, Catalano RB, Paul AR, Bellet RE, Creech RH (1979) Phase II trial of ICRF-159 in previously treated patients with head and neck carcinoma (abstract). Proc Am Soc Clin Res 20:366a
28. Smith MA, Ungerleider RS, Horowitz ME, Simon R (1991) Influence of doxorubicin dose intensity on response and outcome for patients with osteogenic sarcoma and Ewing's sarcoma. J Natl Cancer Inst 83:1460
29. Somlo G, Doroshow JH, Forman SJ, Leong LA, Margolin KA, Morgan RJ Jr, Raschko JW, Akman SA, Ahn C, Schmidt GM, Brent J, Rincon A, Nagasawa S (1994) High-dose doxorubicin, etoposide, and cyclophosphamide with stem cell reinfusion in patients with metastatic or high-risk primary breast cancer. Cancer 73:1678
30. Speyer JL, Green MD, Kramer E, Rey M, Sanger J, Ward C, Dubin N, Ferrans V, Stecy P, Zeleniuch-Jacquotte A, Wernz J, Feit F, Meyers M, Slater W, Taubes S, Blum R, Muggia F (1988) Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. N Engl J Med 319:745
31. Speyer JL, Green MD, Zeleniuch-Jacquotte A, Wernz JC, Rey M, Sanger J, Kramer E, Ferrans V, Hochster H, Meyers M, Blum RH, Feit F, Attubato M, Burrows W, Muggia FM (1992) ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. J Clin Oncol 10:117
32. Swain SM, Whaley FS, Gerber MC, Ewer MS, Bianchine JR, Gams RA (1997) Delayed administration of dexrazoxane provides cardioprotection for patients with advanced breast cancer treated with doxorubicin-containing therapy. J Clin Oncol 15:1333
33. Swain SM, Whaley FS, Gerber MC, Weisberg S, York M, Spicer D, Jones SE, Wadler S, Desai A, Vogel C, Speyer J, Mittelman A, Reddy S, Pendergrass K, Velez-Garcia E, Ewer MS, Bianchine JR, Gams RA (1997) Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. J Clin Oncol 15:1318
34. Swain SM, Whaley FS, Ewer MS (2003) Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer 97:2869
35. Synold TW, Doroshow JH (1996) Anthracycline dose intensity: clinical pharmacology and pharmacokinetics of high-dose doxorubicin administered as a 96-hour continuous intravenous infusion. J Infusional Chemother 6:69
36. Synold TW, Spencer M, Doroshow JH (1997) Dexrazoxane potentiates the cytotoxic activity of topotecan in a sequence and schedule dependent manner (abstract). Proc Am Assoc Cancer Res 38:2161a
37. Tetef ML, Synold TW, Chow W, Leong L, Margolin K, Morgan R, Raschko J, Shibata S, Somlo G, Yen Y, Groshen S, Johnson K, Lenz H-J, Gandara D, Doroshow JH (2001) Phase I trial of 96-hour continuous infusion of dexrazoxane in patients with advanced malignancies. Clin Cancer Res 7:1569
38. Von Hoff DD, Layard MW, Bassa P, Davis HL Jr, Von Hoff AL, Rozencweig M, Muggia FM (1979) Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91:710
39. Von Hoff DD, Howser D, Lewis BJ, Holcenberg J, Weiss RB, Young RC (1981) Phase I study of ICRF-187 using a daily for 3 days schedule. Cancer Treat Rep 65:249
40. Wadler S, Green MD, Basch R, Muggia F (1987) Lethal and sublethal effects of the combination of doxorubicin and the bisdioxopiperazine, (+)-1,2,-bis(3-5-dioxopiperazinyl-1-yl) propane (ICRF 187), on murine sarcoma S180 in vitro. Biochem Pharmacol 36:1495
41. Woods WC, Budman DR, Korzun AH, Cooper MR, Younger J, Hart RD, Moore A, Ellerton JA, Norton L, Ferree CL, Colangelo-Bellow A, Frei M, Henderson IC (1994) Dose and dose intensity of adjuvant chemotherapy for stage II, node-positive breast carcinoma. N Engl J Med 330:1253