Phase I Trial of 96-Hour Continuous Infusion of Dexrazoxane in Patients with Advanced Malignancies[1]

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ABSTRACT

Dexrazoxane is a bidentate chelator of divalent cations. Pretreatment with short infusions of dexrazoxane prior to bolus doxorubicin has been shown to lessen the incidence and severity of anthracycline-associated cardiac toxicity. However, because of rapid, diffusion-mediated cellular uptake and the short plasma half-life of dexrazoxane, combined with prolonged cellular retention of doxorubicin, dexrazoxane may be more effective when administered as a continuous infusion. Thus, a Phase I pharmacokinetic trial of a 96-h infusion of dexrazoxane was performed. Dexrazoxane doses were escalated in cohorts of 3 to 6 patients per dose level. All patients received granulocyte-colony stimulating factor at a dose of 5 μg/kg/day starting 24 h after completion of the dexrazoxane infusion. Plasma samples were collected and analyzed for dexrazoxane by high-performance liquid chromatography. Urine collections were performed at baseline and during the infusion to determine the renal clearance of dexrazoxane and the excretion rate of divalent cations. Twenty-two patients were enrolled at doses ranging from 125 to 250 mg/m^2/day. Grade 3 and 4 toxicities included grade 4 thrombocytopenia in 2 patients treated at 250 mg/m^2/day, grade 3 thrombocytopenia and grade 4 nausea and vomiting in 1 patient treated at 221 mg/m^2/day, grade 4 diarrhea and grade 3 nausea and vomiting in 1 patient treated at 221 mg/m^2/day, and grade 3 hypertension in 1 patient treated at 166.25 mg/m^2/day. Steady-state dexrazoxane levels ranged from 496 μg/l (2.2 μM) to 1639 μg/l (7.4 μM). Dexrazoxane plasma CL_{ss} and elimination $t_{1/2}$ were 7.2 ± 1.6 l/h/m^2 and 2.0 ± 0.8 h, respectively. The mean percentage of administered dexrazoxane recovered in the urine at steady state was 30% (range, 10–66%). Urinary iron and zinc excretion during the dexrazoxane infusion increased in 12 of 18 and 19 of 19 patients by a median of 3.7- and 2.4-fold, respectively. These results suggest that dexrazoxane as a 96-h infusion can be safely administered with granulocyte-colony stimulating factor at doses that achieve plasma levels that have been demonstrated previously to inhibit topoisomerase II activity and to induce apoptosis *in vitro*. Additional studies will be required to determine whether the combination of continuous infusions of dexrazoxane and doxorubicin would provide enhanced cardioprotection compared with the currently recommended bolus or short infusion schedules.

INTRODUCTION

Dexrazoxane reduces the incidence and severity of cardiomyopathy in women with metastatic breast cancer receiving doxorubicin (1–4). After intracellular hydrolysis, dexrazoxane forms a bidentate chelator that resembles EDTA. The cardioprotective effect of dexrazoxane is, at least in part, related to its interference with the formation of reactive oxygen radicals by chelation of unbound transition metals (5, 6).

The cellular pharmacology of dexrazoxane has been examined in beating, heart myocytes of adult rats (5). Drug uptake of radiolabeled dexrazoxane was found to be extraordinarily rapid, with maximum levels of myocyte-associated radioactivity detected within 60 s of drug exposure; no further increase in intracellular dexrazoxane concentration was observed at longer exposure times. Efflux of the myocyte-associated radioactivity was equally rapid and essentially complete within 1 min. The uptake and efflux of the drug were energy and temperature independent and thus likely to be diffusion mediated.

Clinical antitumor activity of ICRF-159, the racemic form of dexrazoxane, has been demonstrated in patients with a variety of tumor types including non-small cell lung cancer (7), colorectal carcinoma (8, 9), Kaposi's sarcoma (10), non-Hodgkin's lymphoma (11), acute leukemia (12), and head and neck carcinoma (13). The antitumor mechanisms of action of both dexrazoxane and ICRF-159 likely include iron chelation (14), inhibition of DNA synthesis by bifunctional alkylation (15), and inhibition of the enzymatic activity of topoisomerase II (16).

Phase I studies of dexrazoxane have been performed previously using a variety of dosing schedules. Liesmann *et al.* (17) treated patients with doses ranging from 200 to 1500 mg/m^2 as an i.v. bolus daily for 5 days every 3 weeks. Leukopenia was the dose-limiting toxicity, occurring at doses of 800 mg/m^2 and above. Other toxicities included reversible liver function abnor-

Received 8/16/00; revised 3/14/01; accepted 3/16/01.
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[1] Supported by NIH Grant U01 CA 62505, NIH Grant 5P30 CA 33572, and Pharmacia-Upjohn (Kalamazoo, MI).
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malities, mild nausea and vomiting, low-grade fever, and alopecia. The recommended dose for further study was 800 mg/m^2 i.v. daily for 5 days in heavily pretreated patients and 1250 mg/m^2 i.v. daily for 5 days in less heavily pretreated patients. Von Hoff *et al.* (18) delivered dexrazoxane in doses from 500 to 1500 mg/m^2 as an i.v. bolus daily for 3 days, repeated every 28 days. The dose-limiting toxicity in this trial was myelosuppression, with moderate to severe leukopenia and thrombocytopenia. Nonmyelosuppressive effects were comparable with those reported by Liesmann *et al.* (17).

A Phase I study of dexrazoxane given by 48-h continuous i.v. infusion was performed by Koeller *et al.* (19). The total dose ranged from 200 to 1000 mg/m^2/48 h with treatment repeated every 3–4 weeks. Myelosuppression, particularly granulocytopenia, was dose limiting. Thrombocytopenia occurred in a small number of patients. Mild nausea, malaise, and alopecia were the only nonhematological toxicities encountered. The authors recommended a starting dose for Phase II trials of 1000 mg/m^2 by a 48-h continuous i.v. infusion.

On the basis of the rapid, diffusion-mediated cellular uptake and short plasma half-life of dexrazoxane, combined with prolonged cellular retention of doxorubicin, we postulated that the cardioprotective and potential antineoplastic activity of dexrazoxane might be improved by prolonged infusion. Furthermore, because prior studies have demonstrated the effectiveness of 96-h infusions in diminishing the cardiac toxicity of doxorubicin (20, 21), we hypothesized that it would be useful for future trials to examine the feasibility of delivering dexrazoxane over a similar time frame. Therefore, this Phase I study evaluated the maximally tolerated dose and pharmacokinetics of dexrazoxane administered as a 96-h continuous i.v. infusion in patients with advanced malignancies. Therapy with G-CSF[3] was given to all patients because myelosuppression was an established dose-limiting toxicity of this agent in previous trials.

MATERIALS AND METHODS

Patient Eligibility. All patients entered into this study had histologically proven cancer that was considered refractory to standard therapy or for which no standard therapy existed, a Karnofsky performance status of 60% or better, and an estimated survival of at least 2 months. Patients were also required to have adequate bone marrow function defined as a neutrophil count of at least 1,500/μl, a platelet count of at least 120,000/μl, and adequate renal function defined as a creatinine ≤1.5 mg/dl or creatinine clearance ≥60 ml/min. For patients without liver metastases, bilirubin was required to be no greater than 1.5 mg/dl, and aspartate aminotransferase and alanine aminotransferase less than three times the upper institutional limit of normal; for patients with known liver metastases, bilirubin could not exceed 3.0 mg/dl, and aspartate aminotransferase and alanine aminotransferase were less than four times the upper limit of normal. Patients must have recovered from the toxicities of any prior chemotherapy or radiation therapy. Prior radiation therapy to >25% of the bone marrow was an exclusion criterion, as was prior nitrosourea therapy. All patients provided informed, voluntary consent and signed an informed consent document approved by the Institutional Review Boards of the participating institutions.

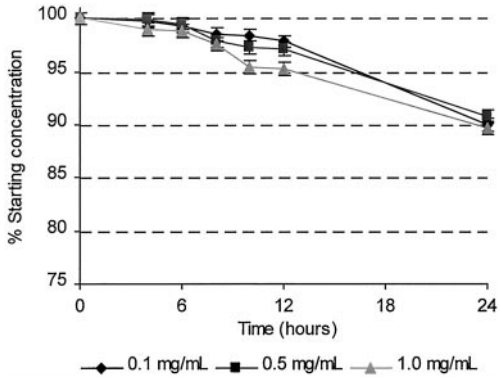

Fig. 1 Stability of dexrazoxane in 5% dextrose.

Pretreatment Evaluation. Pretreatment evaluation included a complete history and physical examination, complete WBC count with differential, a chemistry panel that included liver function tests and serum creatinine, serum magnesium level, chest X-ray, electrocardiogram, and urinalysis. A 24-h urine collection for dexrazoxane, iron, zinc, and magnesium clearance was performed prior to the start of therapy and repeated on day 3 of the first infusion. Patients with bidimensionally measurable disease were required to have baseline evaluations within 4 weeks before the first course of therapy. Repeat tumor evaluations were performed after every two cycles of therapy.

Treatment Plan. Dexrazoxane was administered as a 96-h continuous i.v. infusion repeated every 28 days. Treatment was delivered in either the inpatient or the outpatient setting. Prior to the initiation of the clinical trial, a stability study was performed at the City of Hope Analytical Pharmacology Core Facility. Dexrazoxane solutions at concentrations of 0.1, 0.5, and 1.0 mg/ml were prepared in 5% dextrose containing polycarbonate i.v. bags according to the manufacturer's instructions. Solutions were kept in ambient light at room temperature, and aliquots were removed at various time points up to 24 h. Dexrazoxane concentrations were determined using the HPLC assay described below. All determinations were made in triplicate, and the results are depicted in Fig. 1. As shown in the figure, dexrazoxane was found to be ≥90% of its starting concentration for up to 24 h in dextrose solutions containing either 0.1 or 0.5 mg/ml. Therefore, all patients received four consecutive 24-h infusions with their total daily dose diluted to ≤0.5 mg/ml in 5% dextrose.

The initial dose of dexrazoxane was 125 mg/m^2/day. The dose was escalated in cohorts of patients following a modified Fibonacci scheme to 166.25 and 250 mg/m^2/day and then de-escalated to 221 mg/m^2/day when toxicity was encountered. G-CSF was administered at a dose of 5 μg/kg as a single s.c. injection daily starting 24 h after the end of the dexrazoxane infusion and continued until the WBC count exceeded 10,000/

[3] The abbreviations used are: G-CSF, granulocyte-colony stimulating factor; HPLC, high-performance liquid chromatography; AUC, area under the curve.

Table 1 Patient characteristics

Total patients entered	22
Age (yr)	
Median (range)	57 (46–76)
Gender (number, %)	
Women	8 (36%)
Men	14 (64%)
Karnofsky Performance Status	
60%	2
70%	6
80%	8
90%	5
100%	1
Prior therapy	
Surgery	22
Chemotherapy	21
Radiotherapy	12

Table 2 Number of patients with dose-limiting toxicity observed during the first cycle of dexrazoxane

Toxicity	Grade	125[a]	166.25[a]	221[a]	250[a]
Platelets/μl					
25.0–49.9	3	0	0	1	0
<25.0	4	0	0	0	2
Nausea and vomiting	3	0	0	1	0
Nausea and vomiting	4	0	0	1	0
Diarrhea	4	0	0	1	0
Hypertension	3	0	1	0	0

[a] mg/m^2/day × 4 days.

μl. Escalation of the dose of dexrazoxane for a given patient was not allowed. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 1.0. Patients who experienced any grade 4 toxicity were retreated with a dosage reduction of two levels. Patients with grade 3 nonneutropenic toxicity could be retreated with a dosage reduction of one dose level. Escalation of the dose of dexrazoxane for cohorts of patients was based on the toxicities observed during the first cycle of treatment. A minimum of 3 patients were entered at each dose level. Dose-limiting neutropenia was defined as an absolute neutrophil count <500/μl lasting 5 or more days. If no grade 3 or 4 nonneutropenic toxicity and no dose-limiting neutropenia was observed in the first 3 patients, the dose was escalated one level. If a single patient experienced grade 3 or 4 nonneutropenic toxicity or dose-limiting neutropenia, 3 additional patients were entered at the same dose level before escalation. Dose-limiting toxicity was defined to have occurred at the dose at which 2 patients had grade 3 or 4 nonneutropenic toxicity or dose-limiting neutropenia. Six patients were treated at one dose level below that which produced dose-limiting events.

Evaluation of Toxicity and Efficacy. All patients completed at least one cycle of protocol therapy and were evaluable for toxicity. Efficacy was determined in patients with bidimensionally measurable lesions. Complete response was defined as the disappearance of all objective evidence of disease on two separate measurements at least 4 weeks apart. Partial response was defined as a decrease of ≥50% in the sum of the products of the diameters of the measurable lesion(s), without evidence of new lesions for two consecutive evaluations separated by at least 4 weeks. The same criteria were used whether single or multiple lesions were evaluated. Disease progression was defined as an increase of ≥25% in the area of the measurable lesion(s) over the size at the maximum regression or the appearance of new lesions. Disease not meeting these criteria for response or progression was considered stable.

Pharmacokinetic Studies. For determination of dexrazoxane plasma pharmacokinetics, peripheral blood samples were obtained during the first course of therapy at the following times: prior to the start of the infusion; at 4, 6, 24, 48, 72, and 96 h during the infusion; and then at 10, 20, 40, 60, 120, 360, and 720 min after the end of the infusion. At each time point, 5 ml of blood were collected in vacuum tubes containing sodium heparin. Samples were centrifuged at 1500 × *g* for 10 min, and the plasma was separated. Fifty μl of phosphoric acid (42.5% v/v) were added to each 1 ml of plasma to prevent *ex vivo* hydrolysis of dexrazoxane. Plasma was stored at −70°C until analysis by HPLC. Twenty-four-h urine collections for dexrazoxane, iron, zinc, and magnesium urinary clearances were performed prior to the start of the infusion and on day 3 of the infusion.

Because of the low plasma concentration of dexrazoxane expected when the drug is administered as a 96-h continuous infusion, a new, sensitive HPLC method was required. Therefore, a novel assay using gradient separation was developed specifically for this trial. Prior to HPLC analysis, an acid extraction step was used to remove plasma proteins and other acid-insoluble materials. After addition of ICRF-192 as an internal standard (generously provided by Pharmacia-Upjohn, Kalamazoo, MI), 40 μl of 0.6 M trichloroacetic acid were added to 1 ml of plasma, and the sample was centrifuged at 6000 × *g* for 2 min to pellet the insoluble material. The resulting supernatant was then neutralized by addition of 1 ml of a mixture of tri-*n*-octyamine:trichloro-trifluorothane (21.9:78.1% v/v). The sample was again centrifuged at 6000 × *g* for 2 min. Finally, 70 μl of 0.5 M phosphate buffer were added to 0.5 ml of supernatant to adjust the pH to 7.0, and 100 μl were injected on the column.

The HPLC method consisted of gradient separation across a C_{18} column (150 × 4.6 mm; Phenomenex, Torrance, CA) and UV detection at a wavelength of 209 nm. Mobile phase A was 0.01 M phosphate buffer (pH 4.7) with 0.1 mM 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% B 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 of recovery was 101.1% across the entire range of the standard curve. Inter- and intra-day 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.

A model-independent analysis of the dexrazoxane plasma concentration-*versus*-time data was performed to determine the secondary pharmacokinetic parameters. Dexrazoxane plasma

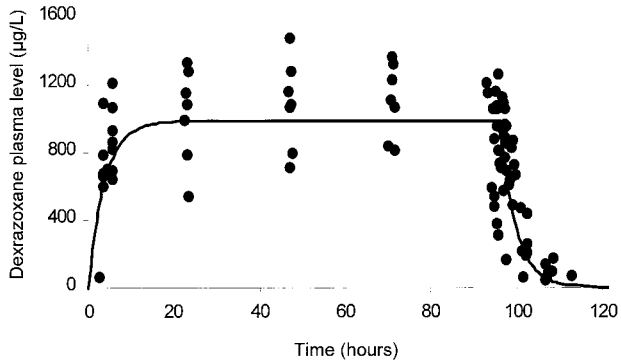

Fig. 2 Dexrazoxane plasma concentration *versus* time plot using pooled data from all patients treated at 166.25 mg/m^2/day ($n = 7$).

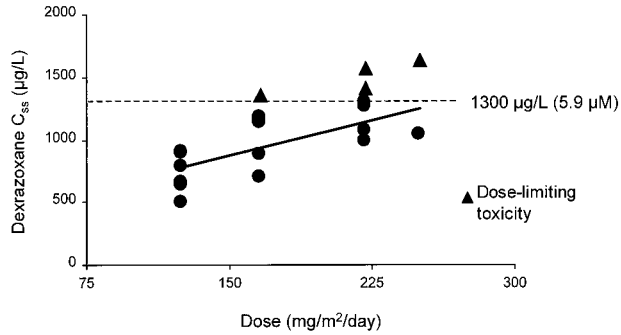

Fig. 3 Steady-state dexrazoxane plasma concentration *versus* administered dose. ▲, patients with dose-limiting hematological toxicity.

Table 3 Dexrazoxane plasma pharmacokinetics

Dose (mg/m^2/day)	CL_{ss} (l/h/m^2)	$t_{1/2}$ (h)	C_{ss} (µg/l)	*AUC* (mg/l × h)
125	5.8	2.1	895.5	80.4
125	5.9	2.3	885.8	74.4
125	8.0	2.0	650.4	60.8
125	6.7	1.6	777.4	70.7
125	8.3	1.4	634.0	61.9
125	10.4	1.2	496.4	47.8
166.25	6.2	2.3	1131.2	102.9
166.25	7.8	2.3	877.3	79.7
166.25	6.0	2.2	1130.3	112.2
166.25	6.1	1.8	1155.7	114.3
166.25	5.8	3.2	1175.3	112.9
166.25	5.1	4.3	1353.7	123.4
166.25	10.0	1.0	699.9	63.1
221	9.3	2.9	988.5	83.3
221	7.3	1.0	1264.8	119.7
221	8.6	2.2	1073.2	100.1
221	6.5	1.6	1407.8	135.2
221	7.1	1.4	1290.3	114.8
221	5.8	1.4	1569.1	151.1
250	6.4	2.4	1639.2	138.3
250	10.0	0.9	1040.5	95.5
Mean	7.2	2.0		
Median	6.7	2.0		
SD	1.6	0.8		

AUC was estimated by linear trapezoids, with the terminal area extrapolated to infinity using each patient's end of infusion concentration to determine the rate of decay (K_{el}). Elimination half-life ($t_{1/2}$) was defined as $0.693/K_{el}$, and dexrazoxane steady-state plasma concentrations (C_{ss}) were determined by taking the average of the levels measured at 48, 72, and 96 h during the infusion. Dexrazoxane clearance at steady-state (CL_{ss}) was determined from the equation:

$$CL_{ss} = (\text{Total dose/Duration of infusion})/C_{ss}.$$

RESULTS

Patient Characteristics. Of the 22 patients entered into this study, there were 8 with colorectal cancer (36%), 4 with breast cancer (18%), 2 with nasopharyngeal cancer (9%), 2 with lung cancer (9%), and 6 with primary disease at other sites. The

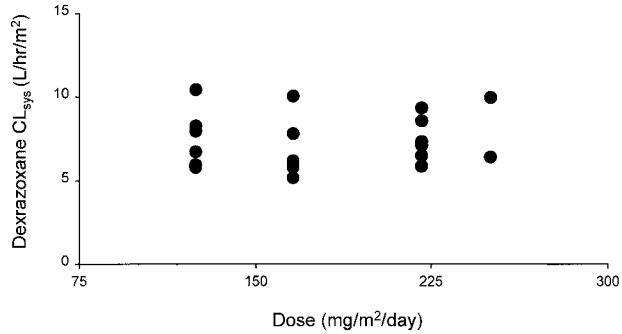

Fig. 4 Dexrazoxane plasma clearance *versus* administered dose.

demographic features of the patients are shown in Table 1. Of the 22 patients, all had prior surgical resections, and 21 had received chemotherapy prior to study entry.

Toxicity. All toxicities for which there was at least one drug-related occurrence graded 3 or higher are shown in Table 2. No toxicities of more than grade 2 were observed at a dose of 125 mg/m^2/day. A dose of 250 mg/m^2/day produced grade 4 thrombocytopenia in 2 patients. At 221 mg/m^2/day, one patient developed grade 4 diarrhea and grade 3 nausea and vomiting, whereas a second patient treated at that level developed grade 3 thrombocytopenia and grade 4 nausea and vomiting. Of note, however, is that both of these patients developed this gastrointestinal toxicity concurrent with bowel obstructions secondary to progressive colon cancer, so that in both patients the gastrointestinal symptoms did not appear to be a direct toxicity of dexrazoxane. One patient treated at 166.25 mg/m^2/day developed grade 3 hypertension; however, this patient did have a long-standing history of hypertension; therefore, it is unlikely that the blood pressure elevation was a direct result of treatment.

Dexrazoxane Pharmacokinetics. Dexrazoxane pharmacokinetic studies were performed in 21 patients: 6 at 125 mg/m^2/day, 7 at 166.25 mg/m^2/day, 6 at 221 mg/m^2/day, and 2 at 250 mg/m^2/day. The pharmacokinetic behavior of infusional dexrazoxane in the 7 patients treated with 166.25 mg/m^2/day is shown in Fig. 2. Dexrazoxane C_{ss} is achieved within the first 12 h of the infusion and is maintained for the remainder of the 96 h. Upon completion of the infusion, plasma dexrazoxane

Table 4 Urinary excretion of iron and zinc

Dose (mg/m^2/day)	Pre-tx[a] Fe^{2+} (μg/24 h)	Day 3 Fe^{2+} (μg/24 h)	Fold change	Pre-tx[a] Zn^{2+} (μg/24 h)	Day 3 Zn^{2+} (μg/24 h)	Fold change
125	184.0	779.0	3.2	62.3	2228.2	34.8
125	114.0	444.0	2.9	219.1	1252.1	4.7
125	77.0	970.0	11.6	520.5	2070.9	3.0
125	10.0	205.0	19.5	401.0	1418.2	2.5
125	84.0	44.0	−0.5	727.7	2249.5	2.1
166.25	14.0	111.0	6.9	726.3	1721.1	1.4
166.25	15.0	78.0	4.2	520.5	1566.6	2.0
166.25	129.0	34.0	−0.7	607.0	2426.2	3.0
166.25	N/A	N/A	N/A	2240.3	2218.2	0.0
166.25	5.0	32.0	5.4	585.2	1618.9	1.8
166.25	28.0	20.0	−0.3	1352.2	3731.3	1.8
166.25	242.0	234.0	0.0	4924.5	7665.3	0.6
221	117.0	44.0	−0.6	713.7	3526.6	3.9
221	10.0	100.0	9.0	650.2	2229.1	2.4
221	7.0	85.0	11.1	473.4	3403.8	6.2
221	19.0	104.0	4.5	495.5	3569.0	6.2
221	64.0	64.0	0.0	1381.2	1913.4	0.4
250	10.0	261.0	25.1	329.0	2192.2	5.7
250	10.0	152.0	14.2	1031.7	2423.7	1.3
Mean	69.9	209.3	4.8	976.5	2635.8	4.5
Median	46.0	92.5	3.7	607.0	2228.2	2.4
SD	69.8	266.1	7.5	1083.3	1432.2	7.6

[a] Pre-tx, pretherapy.

concentrations decline rapidly in a mono-exponential fashion and are undetectable within 12–24 h. Mean pharmacokinetic parameters derived from the model-independent analysis are given in Table 3. The mean (SD) dexrazoxane CL_{ss} and $t_{1/2}$ were 7.2 l/h/m^2 (1.6 l/hr/m^2) and 2.0 h (0.8 h), respectively. The mean percentage of administered dexrazoxane recovered in the urine at steady state was 40.5% (range, 15.6–80.6%).

The dexrazoxane C_{ss} measured ranged from 496 μg/l (2.2 μM) to 1639 μg/l (7.4 μM). As shown in Fig. 3, C_{ss} increased linearly with dose, indicating that the pharmacokinetics of dexrazoxane are linear over the dose ranges studied. This is illustrated further in Fig. 4, which demonstrates that dexrazoxane CL_{ss} is independent of dose. Moreover, the ranges of C_{ss} and CL_{ss} are similar at each of the four dose levels studied (an ~2-fold dose range).

Dexrazoxane Pharmacodynamics. In addition to urinary excretion of dexrazoxane, excretion of the divalent cations Fe^{2+}, Zn^{2+}, and Mg^{2+} were measured prior to and during the infusion on day 3. Table 4 contains the urinary excretion data for all patients studied, whereas Fig. 5 depicts the changes seen in urinary excretion of these metals with dexrazoxane treatment. Compared with pretreatment values, Fe^{2+} and Zn^{2+} excretion increased in 12 of 18 and 19 of 19 patients studied by a median of 3.7- and 2.4-fold, respectively. Mean pretreatment Fe^{2+} and Zn^{2+} were 70 μg/24 h and 976 μg/24 h, increasing to means of 209 μg/24 h ($P = 0.01$) and 2636 μg/24 h ($P < 0.0001$). The magnitude of the change in excretion of Fe^{2+} and Zn^{2+} was unrelated to dexrazoxane dose, C_{ss}, or renal clearance. Urinary excretion of Mg^{2+} was unchanged.

Although dexrazoxane C_{ss} was not predictive of urinary metal excretion, drug levels did correlate with the occurrence of dose-limiting toxicity. As shown in Fig. 3, all patients (4 of 4) who experienced dose-limiting toxicity had dexrazoxane C_{ss} >1300 μg/l (~6 μM). In contrast, none of the patients (0 of 17) with a C_{ss} <1300 μg/l experienced a dose-limiting toxicity, including 2 patients with C_{ss} of 1245 and 1290 μg/l.

Effect of Treatment and Response to Therapy. The number of cycles administered to each cohort of patients is listed in Table 5. One patient treated at dose level 166.25 mg/m^2/day with metastatic breast cancer developed rapid disease progression with substantial decline in performance status during therapy. This patient was felt to be inevaluable for toxicity and was replaced by one additional patient at that dose level. Twelve patients received one cycle of therapy, five received two cycles, two received three cycles, and three received four cycles. There were no objective responses in the 21 evaluable patients.

DISCUSSION

Although dexrazoxane is an effective cardioprotective agent when administered as a short infusion immediately prior to doxorubicin, no data exist regarding its efficacy when used as a continuous infusion combined with infusional doxorubicin. Because it has been demonstrated that the incidence of anthracycline-induced cardiotoxicity is significantly decreased by administering the anthracycline as a 96-h continuous infusion (20, 21), the rationale for the current study was to identify the MTD of infusional dexrazoxane that could be combined with doxorubicin as concurrent 96-h infusions.

The MTD of dexrazoxane when given as a 96-h continuous infusion is 665 mg/m^2/96 h, consistent with the recommended dose delivered by short infusion (500–750 mg/m^2) in combination with conventional doses of doxorubicin. The mean dexrazoxane C_{ss} at the MTD is 1075 μg/l (4.9 μM). Thrombocytopenia, gastrointestinal toxicity, and hypertension were the dose-limiting toxicities of dexrazoxane administered by 96-h continuous i.v. infusion followed by G-CSF support. In prior

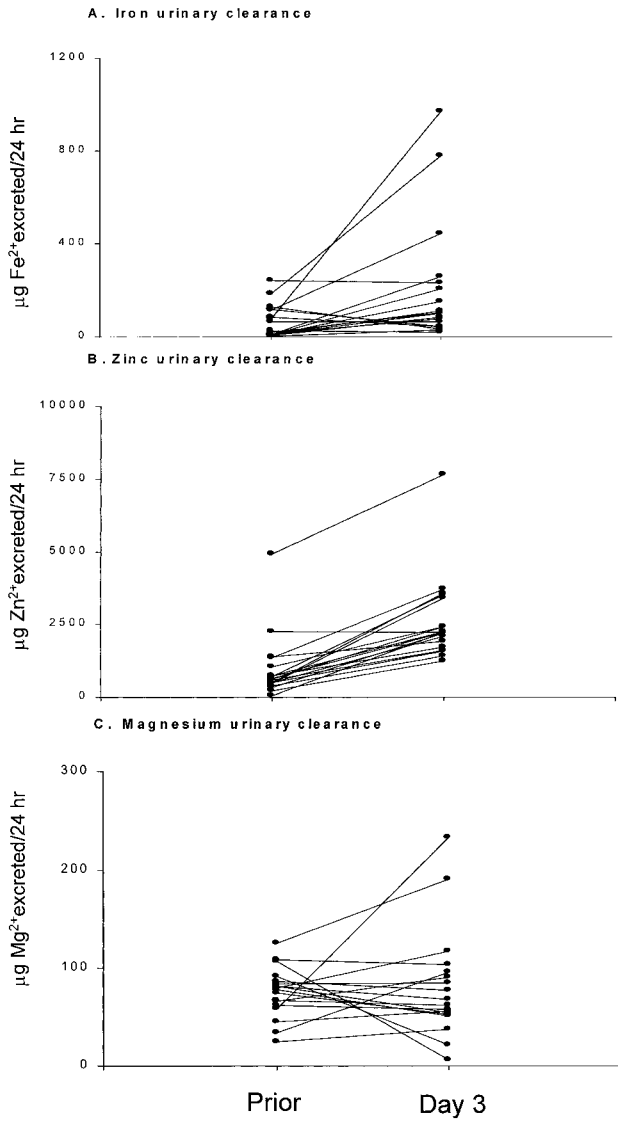

Fig. 5 Urinary clearance of iron (*A*), zinc (*B*), and magnesium (*C*) prior to the start and on the third day of a 4-day dexrazoxane infusion.

studies of dexrazoxane given as a short i.v. infusion, myelosuppression (particularly thrombocytopenia) was the major toxicity. The gastrointestinal toxicity and hypertension observed in our patients were most likely attributable to unrelated medical problems; however, a direct effect of dexrazoxane cannot be excluded. Interestingly, we observed a sharp increase in the occurrence dose-limiting toxicities when the C_{ss} exceeded 1300 μg/l (5.9 μM), suggesting a steep dose-response relationship.

The recommended dose of dexrazoxane when used in combination with doxorubicin is 10 times the doxorubicin dose on a mg per mg basis. Although the MTD (on a mg basis) of dexrazoxane for the current trial is only four times the dose of doxorubicin used in current City of Hope bone marrow transplant trials (165 mg/m^2), pharmacokinetic studies demonstrate that steady-state plasma levels of dexrazoxane at the MTD are ~18 times greater than doxorubicin (22). *In vitro* studies demonstrate complete cardioprotection of rat heart myocytes from doxorubicin-induced heart damage if dexrazoxane is present in 10-fold excess (5). Therefore, it is likely that dexrazoxane given at a dose of ~166 mg/m^2/day will be effective as a cardioprotective agent in combination with infusional doxorubicin at doses of up to 165 mg/m^2/96 h.

Table 5 Number of cycles administered

	Dexrazoxane dose (mg/m^2/day × 4 days)				
	125	166.25	221	250	Total
1 cycle	4	2	5	1	12
2 cycles	1	2	0	2	5
3 cycles	1	1	0	0	2
4 cycles	0	2	1	0	3
Total	6	7	6	3	22

The plasma pharmacokinetics of dexrazoxane by a number of different infusion schedules has been studied previously (23–25), and the pharmacokinetic data from the current trial are in good general agreement with prior results. Earhart *et al.* (23) studied the pharmacokinetics of 1000 mg/m^2 dexrazoxane given as either a 30-min, 8-h, or 48-h infusion. The investigators concluded that the systemic clearance of dexrazoxane was not dependent on the infusion rate. The mean dexrazoxane C_{ss} achieved when 1000 mg/m^2 was delivered over 48 h was 2900 μg/l, compared with 1340 μg/l when the same total dose was given over 96 h on the current study. These data taken together suggest that the pharmacokinetics of dexrazoxane are linear over a very wide range of doses and schedules. Urinary recovery of dexrazoxane has been reported to be 48% of the administered dose (23), compared with 41% on the current trial.

Von Hoff *et al.* (18) reported that urinary excretion of Fe^{2+} and Zn^{2+} were increased 10-fold during treatment with dexrazoxane, whereas clearance of Mg^{2+}, Ca^{2+}, and Cu^{2+} were unchanged. The current study confirms these results, with mean increases in Fe^{2+} and Zn^{2+} urinary clearance of 4.8- and 4.5-fold, respectively. The smaller increase in urinary metal excretion is likely attributable to a greater amount of dexrazoxane being administered over a shorter time period when given as a short rather than a prolonged infusion. In the current study, no relationship between dexrazoxane exposure and urinary clearance of metals could be identified over the fairly small dose and C_{ss} range studied (2- and 3-fold, respectively). Moreover, there was no association between the change in either Fe^{2+} or Zn^{2+} clearance and the occurrence of dose-limiting toxicity. The mean urinary excretion of Fe^{2+} during a 96-h continuous infusion of dexrazoxane is 209 ± 266 μg/24 h or 836 μg/96 h, assuming a constant rate.

Dexrazoxane was originally investigated as an anticancer agent in the 1970s and 1980s. Most of the single-agent studies of dexrazoxane investigated the use of short infusions given daily for 3–5 days (15, 17, 18). Interestingly, the MTDs of dexrazoxane determined using these short infusion schedules are approximately 10–15 times higher than the MTD determined on the current study using a 96-h continuous infusion schedule. In one Phase I study of dexrazoxane given by 48-h continuous infusion (19), the MTD is ~2-fold higher than that of the current study. When combined, these clinical data provide

strong evidence for schedule-dependent dexrazoxane toxicity *in vivo*.

The discovery that dexrazoxane inhibits the catalytic activity of topoisomerase II (16, 26, 27) has renewed interest in this compound as an antitumor agent. However, there is a paucity of *in vitro* data regarding the cytotoxic effects of prolonged exposures of dexrazoxane. The limited data that exist ignore the significant chemical instability of dexrazoxane under tissue culture conditions (28). Once added to culture medium, dexrazoxane is rapidly converted to its ring-opened chelating form (29, 30). It has been determined that dexrazoxane itself, and not its hydrolysis products, is responsible for tumor cell killing *in vitro*. Hasinoff *et al.* (31) have shown that whereas dexrazoxane inhibits topoisomerase II, its ring-opened hydrolysis products do not. High millimolar concentrations of dexrazoxane hydrolysis products are required *in vitro* for cytotoxicity,[4] compared with micromolar concentrations for the parent compound. Schedule-dependent cytotoxicity observed both *in vivo* and *in vitro* is consistent with evidence suggesting that topoisomerase II is a target for dexrazoxane. Moreover, given the steep dexrazoxane C_{ss}-toxicity relationships observed in this study, inhibition of topoisomerase II by dexrazoxane may be an all-or-nothing phenomenon.

Because of the lack of *in vitro* cytotoxicity data for prolonged dexrazoxane exposures, we undertook a series of laboratory studies to provide a context for the plasma levels measured in this clinical trial. However, because of the instability of dexrazoxane in tissue culture medium ($t_{1/2}$, ~3 h), a method for maintaining constant drug levels was required. As reported previously (28), we have developed methodology allowing us to control drug levels for >96 h using ALZET osmotic pumps (ALZA Scientific, Palo Alto, CA) to simulate a continuous infusion *in vitro*. By applying this procedure, we demonstrated that the levels of dexrazoxane achievable *in vivo* by a 96-h infusion schedule (~5 μM) are above the concentrations required to induce cytotoxicity in K562 cells, an erythroleukemic cell line (IC_{50}, 3.6 μM; Ref. 32).

The Phase I study described here is the first trial of dexrazoxane by 96-h continuous infusion. It is now clear from clinical and laboratory investigations that dexrazoxane acts both as a cardioprotective and a cytotoxic agent. The pharmacokinetic analyses performed in support of this trial have identified that steady-state dexrazoxane concentrations in the range of 3–5 μM for 96 h are achievable *in vivo* without occurrence of unacceptable toxicity. In light of these data, new trials of dexrazoxane in combination with anthracyclines and other chemotherapeutic agents are now being designed to take advantage of the unique pharmacology of dexrazoxane. For example, the results of the current study have been used to design a trial combining simultaneous 96-h continuous infusions of dexrazoxane with high-dose doxorubicin that is ongoing at the City of Hope. It is hypothesized that giving both agents as concurrent 96-h infusions will decrease the cardiotoxicity associated with high-dose doxorubicin. We anticipate that this combination will allow us to administer dose-intensive doxorubicin more safely to patients who have received substantial doses of anthracycline prior to high-dose therapy. Moreover, because of the clinical interest in the combination of topoisomerase I and II inhibitors, two Phase I trials were initiated at the City of Hope for patients with hematological malignancies and solid tumors that combine dexrazoxane and the topoisomerase I inhibitor, topotecan. Given the favorable safety profile of infusional dexrazoxane and the demonstration that μM concentrations are sustainable for long periods *in vivo*, it is likely that the role of dexrazoxane will expand as it develops as both a cardioprotectant and an antineoplastic agent.

[4] B. Hasinoff, personal communication.

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Clin Cancer Res 2001;7:1569-1576.