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ORIGINAL ARTICLE

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Phase I clinical trial of 7-cyanoquinocarcinol (DX-52-1) in adult patients with refractory solid malignancies

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Abstract *Purpose*: A phase I study of the antitumor antibiotic 7-cyanoquinocarcinol, DX-52-1, was conducted in patients with refractory solid malignancies. This study sought to determine the maximum tolerated dose and principal toxicities of this agent and to characterize its pharmacokinetic behavior. *Methods*: Patients were required to have adequate bone marrow, renal and hepatic function. DX-52-1 was administered by i.v. continuous infusion over a 6-h period each week for four consecutive weeks followed by a 2-week rest period, which constituted one cycle of treatment. *Results*: Initial dose levels were 3, 6, and 10 mg/m^2. An intermediate dose level of 8 mg/m^2 was added after acceptable toxicity was observed at the 6 mg/m^2 dose level, but dose-limiting toxicities, including life-threatening ones, were seen at the 10 mg/m^2 dose level in all three patients. The maximum tolerated dose (MTD) was subsequently determined to be 6 mg/m^2. Because a clear pattern of toxicities was not initially evident, a larger than usual number of additional patients (16) were enrolled at the MTD to better distinguish toxicities due to the study drug from those secondary to the patients' underlying malignancies. Even at the MTD, the drug was poorly tolerated, with gastrointestinal toxicities (abdominal pain, nausea, vomiting and increased liver function tests) predominating and dose-limiting. Pharmacokinetic studies revealed that the mean maximum plasma concentration of DX-52-1 in patients evaluated at the MTD (138.8 ± 59.3 ng/ml, $n = 19$) was considerably lower than the concentrations required for cytostatic or cytotoxic activity against sensitive human tumor cell lines in vitro. Further, the weekly dose intensity of the most efficacious treatment schedule identified during in vivo antitumor efficacy studies was 60 times greater than the 6 mg/m^2 weekly dose tolerated by cancer patients. None of the 33 patients participating in this study, including the 22 patients evaluated at the MTD, had any response to treatment. *Conclusion*: Given the poor tolerability, the inability to achieve drug levels necessary to inhibit in vitro or in vivo tumor growth, and the lack of any responses in our study, DX-52-1, as given by this schedule, does not appear to warrant further investigation in phase II studies.

Keywords DX-52-1 · Quinocarmycin · Cancer · Chemotherapy · Clinical trials · Pharmacokinetics · Human

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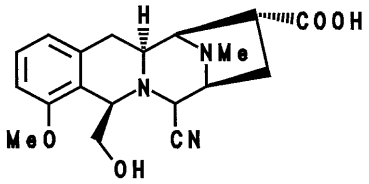
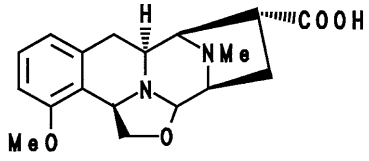
Introduction

Quinocarmycin (Fig. 1) is an antitumor antibiotic produced by *Streptomyces melanovinaceus* [23, 24]. It belongs to the naphthyridinomycin/saframycin class of antitumor antibiotics, characterized by the tetracyclic hexahydropyrazinoisoquinoline ring system, but is structurally simpler than any other naturally occurring member of this family [26]. Quinocarmycin demonstrated promising growth inhibitory activity against a variety of human tumor cell lines in vitro and human tumor xenograft models in vivo [2, 6]. A limited clinical evaluation of the citrate salt of quinocarmycin was initiated in Japan [7, 16]. However, the semisynthetic derivative 7-cyanoquinocarcinol (DX-52-1, Fig. 1) was considered to be a more suitable candidate for clinical development due to superior chemical stability than the parent compound in aqueous solution [16, 18, 19].



DX-52-1



QUINOCARMYCIN

Fig. 1 Chemical structures of DX-52-1 and quinocarmycin

DX-52-1 and quinocarmycin were among the first agents identified by the NCI disease-oriented human tumor cell line screen to exhibit a high level of specificity for melanoma [16]. When evaluated at the LC_{50} level of effect, seven of the eight cell lines in the melanoma panel were markedly more sensitive to DX-52-1 than the average of all 53 cell lines comprising the screen. Although *N*-heterocyclic natural products are paradigmatic substrates of the transmembrane transport proteins associated with multidrug resistance [10], DX-52-1 was found to be more active against a multidrug-resistant subline of murine P388 leukemia developed through exposure to Adriamycin than the parent cell line [16]. The lack of recognition by the multidrug-resistance phenotype augmented the rationale for continued development of the agent as a potential clinical candidate.

The exceptional antiproliferative activity of DX-52-1 was corroborated by demonstrating notable in vivo efficacy against staged subcutaneously implanted tumor xenografts derived from several of the human melanoma cell lines [16]. Inhibition of tumor growth achieved with the optimal dosing regimen of DX-52-1 (three to six treatments administered by the intraperitoneal route at 4-day intervals) exceeded the effects achieved with 11 of 12 standard clinical anticancer drugs, with 1,3-*bis*(2-chloroethyl)-1-nitrosourea being the only exception. Preclinical toxicology studies indicated that higher i.v. doses of the drug were tolerated when administered slowly as an infusion rather than as a rapid injection [20]. Impairment of the kidneys, intestinal epithelium, lymphoid organs and bone marrow were the principal effects observed in animals treated with toxic doses of the drug.

The underlying mechanism by which the quinocarmycins induce cytotoxicity has not been conclusively established. Nevertheless, a compelling argument based upon experimental findings and several lines of indirect evidence has been advanced to suggest that they act by alkylating DNA in the same manner as demonstrated for naphthyridinomycin, saframycin A, and ecteinascidin-743 [5, 14, 17, 19]. The pattern of differential cytotoxicity of DX-52-1 against the cell lines comprising the NCI anticancer screen most closely resembled DNA binding and alkylating agents, with saframycin analogues providing some of the highest correlation coefficients, when compared to a database of screening results for approximately 8000 compounds [16]. A common structural feature that appears to be required for the biological activity of the saframycins and related compounds is the presence of a good leaving group, such as hydroxyl, cyano or alkoxy substituent, on the piperazine-like ring at the position adjacent to the tetrahydroisoquinoline nitrogen [17]. For example, quinocarcinol is structurally identical to DX-52-1 except for the lack of a leaving group at this position but does not exhibit any antibiotic or antitumor activity [24]. General acid-catalyzed elimination of the leaving group affords the activated form of the drug, a reactive iminium intermediate, which covalently adds to the exocyclic 2-amino group of a guanine residue in the minor groove of DNA [17, 19]. In the case of DX-52-1 and quinocarmycin, loss of cyanide or opening of the oxazolidine ring, respectively, would generate a structurally identical intermediate. It is worth noting that this is analogous to the mechanism originally proposed by Hurley et al. to account for the cytotoxic activity of anthramycin [17].

DX-52-1 was selected for clinical development by the NCI on the basis of its melanoma-specific in vitro cytotoxicity and evidence of encouraging in vivo antitumor activity. This report describes the results of a phase I study of DX-52-1 administered as a 6-h continuous i.v. infusion given once every 7 days for four consecutive weeks to patients with refractory solid tumors. The primary objectives of the study were to identify the principal dose-limiting toxicity (DLT), establish an appropriate dose for phase II trials, and to characterize the pharmacokinetic behavior of the drug.

Materials and methods

Patient selection

Patients with histologically confirmed malignancies that were not curable with surgery, radiation or standard chemotherapy were eligible for the study. Patients were required to have adequate bone marrow function (neutrophil count $\geq 1250/mm^3$, platelet count $\geq 100{,}000/mm^3$), renal function (serum creatinine ≤ 1.6 mg/dl and creatinine clearance ≥ 70 ml/min), hepatic function (aspartate aminotransferase less than four times normal and bilirubin ≤ 1.5 mg/dl), age more than 18 years, an Eastern Cooperative Oncology Group performance status of not more than 2, no serious comorbidity, and no evidence of a myocardial infarction occurring within 6 months. During the course of the study, the selection criteria were amended to exclude patients with a single functioning kidney or history of cisplatin-related nephropathy, following notification by the NCI of an adverse drug event occurring in a patient with these characteristics following treatment with DX-52-1 during a study independently performed at another institution. All patients signed an informed consent which met Federal and institutional requirements prior to entry into the study.

Treatment protocol

DX-52-1 was supplied by the Cancer Treatment Evaluation Program, NCI (Bethesda, Md.). Each 30 ml flint vial of the injectable, provided as a white lyophilized powder, contained 5 mg DX-52-1, 250 mg lactose, 1.35 mg citric acid and 130 mg disodium hydrogen phosphate. Intact vials were stored in a refrigerator at 2–8°C. Reconstitution with 5 ml 0.9% Sodium Chloride Injection USP afforded a 1 mg/ml solution of the drug with a pH of 7.5–8.5. This solution was further diluted for administration to a final drug concentration in the range 10–200 μg/ml with 0.9% Sodium Chloride Injection USP.

All patients underwent complete laboratory studies and disease assessments within 14 days before starting treatment. Patients fully meeting all eligibility requirements were assigned to the appropriate dose level. The drug was administered by continuous i.v. infusion over a 6-h period each week for four consecutive weeks, followed by a 2-week rest period, which constituted one cycle of treatment. The weekly dose of DX-52-1 was escalated from an initial level of 3.0 mg/m^2 according to a modified Fibonacci progression [21]. Patient histories, physical examinations, and repeat laboratory studies were performed on a weekly basis while on study.

Three patients were initially enrolled at each dose level with treatment repeated every 6 weeks as permitted by their conditions. Evaluation of successive dose levels proceeded after all three patients had received the first cycle of therapy with the preceding dose and each was observed for at least 14 days without evidence of a DLT, as defined below. An additional three patients were entered into a given dose level in cases where a single patient experienced a DLT during the first cycle of therapy. The occurrence of a DLT in two patients from any cohort of three to six established the preceding dose level as the maximum tolerated dose (MTD). The tentative MTD was expanded by adding ten additional patients to better estimate the true toxicity rate of the prospective phase II dose.

Drug-related toxicities were evaluated and graded according to the NCI Common Toxicity Criteria [11]. A DLT was defined as any of the following events: grade 3 or greater nonhematologic toxicity, grade 4 neutropenia or thrombocytopenia persisting for at least 4 days, or delay of therapy for more than 28 days after completing the prior 6-week treatment cycle due to out-of-range laboratory values.

Response criteria

Patients were evaluated for therapeutic response after completing every other cycle of therapy. Tumors were measured by radiological methods, ultrasonography, and/or physical examination. Tumor burden was calculated as the sum of the products of the longest perpendicular diameters of all measurable lesions. Tumor measurements were repeated bimonthly, using the same procedure, and continued on a regular basis until relapse. Complete response was defined as the disappearance of all measurable disease, signs, symptoms, and biochemical changes related to the tumor. A reduction in tumor burden of 50% or greater constituted a partial response. Stable disease was defined as a less than 50% decrease in tumor burden or an increase that did not exceed 25%. In addition, for each of these classifications, the response or disease stabilization had to persist for a minimum of 4 weeks during which time no new lesions were detected. Progressive disease was indicated by a greater than 25% increase in tumor burden or the appearance of any new lesion.

Pharmacokinetic sampling and quantitation

Blood specimens were acquired to characterize the plasma pharmacokinetics of DX-52-1 in all patients during administration of the first weekly dose of the initial cycle of therapy. Blood was drawn into Vacutainer Brand plasma tubes containing freeze-dried sodium heparin (Becton Dickinson, Franklin Lakes, N.J.) from a peripheral venous catheter placed in the arm of the patient not used for infusing the drug. Patency of the sampling catheter was maintained between blood draws with a heparin lock or slow running line of Normal Saline For Injection USP. Samples (5 ml) were obtained before treatment and at 9 to 13 predetermined timepoints during the infusion and for 3 h after the infusion had ended. A battery-powered digital timer was used to accurately monitor the beginning and ending times of the infusion and sample collection intervals. The sample tubes were mixed by inversion and placed on ice until centrifuged for 10 min at 2500 *g*. The plasma was pipetted into polypropylene cryovials and stored at –70°C until assayed.

The concentration of DX-52-1 in the plasma specimens was measured by a validated reversed-phase HPLC assay, precisely as reported [22]. Each set of patient samples was assayed in duplicate, on different days, together with a series of eight plasma standards with added DX-52-1 concentrations ranging from 250 to 2.5 ng/ml, and a drug-free sample. Standard curves were constructed by plotting the chromatographic peak area of the drug as a function of the corresponding concentration in plasma. The drug concentrations in patient samples were calculated using the slope and intercept of the line fitted to the standard curve by linear regression performed with a weighting factor of $1/y_{obs}$. Study samples were reassayed in cases where the two initial determinations differed from their average by more than 10%. In addition, specimens with concentrations exceeding the upper limit of the standard curve were reassayed in duplicate upon dilution with drug-free plasma.

The lower limit of quantitation of the assay was 2.56 ng/ml. Within-day accuracy was 98.0–102.3% with a precision of 0.5–4.8% at five concentration levels spanning the entire range of the standard curve. Between-day accuracy and precision of the analytical method were assessed by analyzing the interpolated drug concentrations from 21 standards curves during a 4-month period. The mean between-day accuracy was 101.0 ± 1.6% (SD) and the precision ranged from 2.7% for the 256.0 ng/ml plasma standard to 18.9% at the 2.56 ng/ml limit of quantitation.

Pharmacokinetic data analysis

Actual sample times relative to the beginning of the infusion were calculated and used for the pharmacokinetic analysis of the data. Individual patient plasma concentration-time profiles of the drug were analyzed by noncompartmental methods using the WinNonlin Version 1.1 software package (Scientific Consulting, Apex, N.C.) [4]. Area under the plasma concentration-time curve from time zero to infinity (AUC) was estimated using the linear/log trapezoidal algorithm to the last data point, with extrapolation to infinity by adding the quantity $C(t_n)/\lambda_z$, where $C(t_n)$ is the observed drug plasma concentration at the last measurable time-point and λ_z is the absolute value of the slope of the terminal log-linear data points. The area under the first moment curve was estimated in an analogous manner. These values were used to calculate the apparent biological half-life ($t_{1/2,z}$), mean residence time (MRT), total plasma clearance (CL), and apparent volume of distribution at steady state (V_{ss}) according to standard equations [4].

The mean plasma concentration of DX-52-1 and average value of the actual sample times were calculated at each nominal timepoint using all available data from a total of 19 patients treated at the 6 mg/m^2 dose level. The following model-independent equation for zero-order i.v. drug input and biexponential disposition [15] was fitted to the mean plasma concentration-time curve by weighted nonlinear least squares regression using WinNonlin:

$$C = \frac{C_1}{\lambda_1 T}(e^{-\lambda_1 t'} - e^{-\lambda_1 t}) + \frac{C_z}{\lambda_z T}(e^{-\lambda_z t'} - e^{-\lambda_z t})$$

In this equation, the value of t′ is zero until the infusion of duration T has terminated, upon which it becomes defined as t′ = t–T, where t denotes time relative to the beginning of the infusion. The coefficients C_i are intercept values, corresponding to i.v. bolus administration of the dose, of each log-linear phase with a slope $-\lambda_i$, such that $\lambda_1 > \lambda_z$. Initial estimates of the iterated parameters (C_i, λ_i) were determined automatically by the program and then refined using the Levenberg-Hartley modification of the Gauss-Newton algo-

rithm, with upper and lower boundaries on the parameters specified. The number of exponential terms in the fitted equation and influence of the weighting factor, y_{obs}^{-n} ($n = 0$, 1 or 2), were evaluated to ascertain the best fit of the experimental data. This was assessed by a variety of considerations, which included visual inspection of the predicted profile, residual analysis, values of the Akaike and Schwartz Information Criterion, sum of weighted squared residuals, mean standard deviation, degree of correlation between parameters, and standard errors of the parameter estimates [4]. Weighting according to y_{obs}^{-2} yielded the best fit. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic terms according to standard equations [4].

Mean values of all pharmacokinetic variables were calculated as the geometric mean of the individual values determined by noncompartmental analysis [8, 13]. Standard deviations for the geometric mean values were estimated by the jackknife method [9]. Pearson's sample correlation coefficients (r) were calculated to assess relationships between the C_{max} of DX-52-1 and pretreatment laboratory values associated with drug-eliminating organ function for 19 of the patients evaluated at the MTD. Correlations between the maximum percent change in serum chemistry and hematological values observed during the first cycle of therapy and the C_{max} or weekly dose of the drug were similarly assessed for patients evaluated at all dose levels. The suggestion of a significant correlation, as indicated by $|r| \geq 0.4$, was substantiated by examining a scatter plot of the data and the P-value for the slope of the best-fit line determined by linear regression analysis.

Results

Patient characteristics

Pretreatment characteristics of the 33 patients entered into the study are summarized in Table 1. Approximately one-half (48%) of the patients had received more than two prior chemotherapeutic regimens. A total of 53 courses of DX-52-1 were administered and the median number of courses each patient received was two.

Table 1 Patient characteristics ($n = 33$)

Age (years)	
Median	55
Range	23–75
Sex (n)	
Male	19
Female	14
Performance status (n)	
0	16
1	14
2	3
Primary tumor site (n)	
Adenoid	1
Breast	3
Colorectal	7
Gastrointestinal	8
Lung	6
Leiomyosarcoma	1
Melanoma	1
Neuroblastoma	1
Ovarian	1
Prostate	2
Renal	1
Uterine	1
Prior chemotherapy regimens (n)	
0	2
1	5
2	10
3	7
≥4	9

n, number of patients

Toxicity

DX-52-1 administered as a 6-h continuous i.v. infusion resulted in no allergic or other acute reactions. One patient treated at the second dose level of 6.0 mg/m^2 expired 2 days after receiving the first weekly dose of the drug. The cause of death could not be established, and three additional patients were enrolled at this dose level. As no dose-limiting events occurred in these patients, dose escalation proceeded to the next planned level of 10.0 mg/m^2. The entire cohort of three patients treated with 10.0 mg/m^2 DX-52-1 experienced DLTs consisting of grade 3 abdominal pain, nausea, vomiting, transaminitis and hyperbilirubinemia. One patient also experienced a transient grade 3 elevation in serum creatinine following her second weekly dose. Urinalysis revealed moderate proteinuria, glucosuria, and mild hematuria, although casts were not evident. The creatinine and urinalysis improved to baseline values within 2 weeks of interrupting treatment.

Because the 6.0 mg/m^2 dose level produced mild to moderate toxicity, but life-threatening toxicity occurred at the 10.0 mg/m^2 dose, an intermediate dose of 8.0 mg/m^2 was initiated. Five patients were treated at this dose level, three of whom experienced grade 3 or 4 gastrointestinal toxicities and liver function abnormalities. The MTD was therefore determined to be 6.0 mg/m^2 given every 7 days for four consecutive weeks. Because a clear pattern of toxicities was not initially evident, an additional 16 patients were treated at the MTD of 6.0 mg/m^2 to better distinguish the toxicities due to DX-52-1 from those caused by the patients' underlying malignancies. A total of 22 patients were treated with 35 cycles of therapy at 6.0 mg/m^2. The toxicity data are summarized in Tables 2 and 3. Moderate to severe gastrointestinal symptoms, principally nausea, vomiting and abdominal pain, as well as liver function abnormalities were the most frequently occurring toxicities at all dose levels and were dose-limiting at 8.0 mg/m^2.

Responses

There were no objective antitumor responses to DX-52-1 among the 24 patients who were evaluable for response. Only one patient treated at the 8.0 mg/m^2 dose level exhibited evidence of disease stabilization when evaluated 3 months after the beginning of treatment, but had clearly progressed at the time of the second assessment at 6 months. All other patients exhibited progression at their first disease assessment, 3 months after initiating therapy.

Table 2 Clinical toxicities observed in patients following treatment with DX-52-1

Dose level (mg/m^2)	Total courses administered	Grade 2/3 + 4				
		Abdominal pain	Nausea/vomiting	Diarrhea	Fatigue	Edema
Course 1						
3.0	3	0/0	0/0	0/0	0/0	0/0
6.0	22	2/1	5/0	3/0	0/0	0/0
8.0	5	0/0	1/0	0/0	0/1	0/1
10.0	3	0/1	3/1	0/0	0/0	0/0
All courses						
3.0	6	0/0	0/0	0/0	0/0	0/0
6.0	35	2/1	7/0	3/0	4/0	0/0
8.0	9	0/0	1/0	0/0	0/0	0/1
10.0	3	0/1	3/1	1/1	0/0	0/0

Table 3 Graded changes in serum chemistry and hematological parameters in patients treated with DX-52-1

Dose level (mg/m^2)	Total courses administered	Grade 2/3 + 4						
		Alkaline phosphatase elevation	Transaminitis	Bilirubin elevation	Creatinine elevation	Thrombo-cytopenia	Lymphopenia	Anemia
Course 1								
3.0	3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
6.0	22	1/1	3/1	0/0	1/0	0/0	1/1	0/0
8.0	5	1/1	1/1	1/1	0/0	1/0	0/1	0/0
10.0	3	0/1	0/1	1/1	1/1	0/0	0/1	1/0
All courses								
3.0	6	0/0	0/0	0/0	0/0	0/0	0/0	0/0
6.0	35	1/2	6/2	0/2	2/0	1/2	1/5	0/0
8.0	9	1/1	1/1	1/1	0/0	1/0	0/1	0/0
10.0	3	0/1	0/1	1/1	1/1	0/0	0/1	1/0

Pharmacokinetics

Plasma specimens to characterize the pharmacokinetic behavior of DX-52-1 were obtained from all 33 patients during treatment with the first weekly 6-h i.v. infusion of the drug. The sampling schedule used for the initial 15 patients entered into the study, who were treated with doses of 3.0, 6.0 and 10.0 mg/m^2 ($n = 3$, 9 and 3, respectively), included only two time-points during the administration of DX-52-1, near the midpoint and end of the 6-h infusion. Since this schedule did not allow the AUC to be estimated with acceptable accuracy by the trapezoidal method, the protocol was amended to facilitate specimen collection at 1-h intervals during the infusion. This more intensive sampling schedule was used for pharmacokinetic studies in 13 additional patients treated with doses of 6.0 mg/m^2 and a group of 5 patients at the 8.0 mg/m^2 dose level. There were no pharmacokinetic data available from three patients in the 6.0 mg/m^2 cohort due to problems encountered during sample collection or analysis.

A typical plasma profile determined in a patient treated with 6.0 mg/m^2 of DX-52-1 is shown in Fig. 2A. While the drug concentration in plasma increased rapidly after starting the infusion, it did not appear that steady-state conditions were achieved during the course of the 6-h continuous i.v. infusion in a majority of the patients. Moreover, the individual plasma profiles were generally not well described by the usual linear pharmacokinetic models fitted to the experimental data by nonlinear regression. Nevertheless, mean values of the observed C_{max} of DX-52-1 tended to increase linearly as the dose was escalated ($r = 0.916$), as illustrated in Fig. 3. However, there was a relatively high degree of interpatient variability, which was particularly evident at the 6.0 mg/m^2 MTD. The mean C_{max} for 19 of the 22 patients treated at this dose level was 139 ng/ml with a 42.7% CV. In addition, the range of observed C_{max} values for the MTD cohort (43–263 ng/ml, median 143 ng/ml) completely encompassed the C_{max} achieved in the 11 patients treated at each of the other three dose levels (range 44–232 ng/ml).

Mean values of the pharmacokinetic parameters estimated by noncompartmental analysis of 12 individual DX-52-1 plasma profiles for the second group of patients evaluated at the 6.0 mg/m^2 dose level are presented in Table 4. The $t_{1/2,z}$ and MRT were very similar, being 1.52 ± 1.03 h and 1.23 ± 0.46 h, respectively. The CL of the drug was 7.43 ± 1.41 l/h per m^2 and the V_{ss} (9.53 ± 2.98 l/m^2) was relatively small, only 26% of body weight for normal adults. In comparison to the other pharmacokinetic parameters, there was greater interpatient variability in the $t_{1/2,z}$ of DX-52-1, for which the CV was 68%. This is most likely attributable to the inability to monitor plasma levels of the drug for a sufficient period of time to accurately and consistently define the terminal disposition phase due to practical limita-

tions imposed on the duration of postinfusion sampling by performing this study on an outpatient basis. The sensitivity of the analytical method was not a limiting factor as the concentration of drug remained well above the 2.5 ng/ml limit of quantitation in the last plasma specimens acquired from the patients. With outpatient clinics operating on a 10-h day, samples could only be collected for up to 3 h after completing the 6-h drug infusion under the best of circumstances. In actuality, the last pharmacokinetic sample scheduled for collection at 9 h after beginning the infusion was obtained from only 55% of the 33 patients evaluated during the course of this study. Reference to the plasma profiles of DX-52-1 shown in Fig. 2 clearly indicates that the terminal exponential phase cannot be reliably established without this data point. Estimated values of the other pharmacokinetic parameters were relatively insensitive to the terminal phase since the average contribution of the area extrapolated beyond the last data point was only 7.8 ± 3.2% of the AUC and 19.4 ± 8.2% of the AUMC.

Despite the inability to fit the individual patient plasma profiles to a linear pharmacokinetic model, a marked

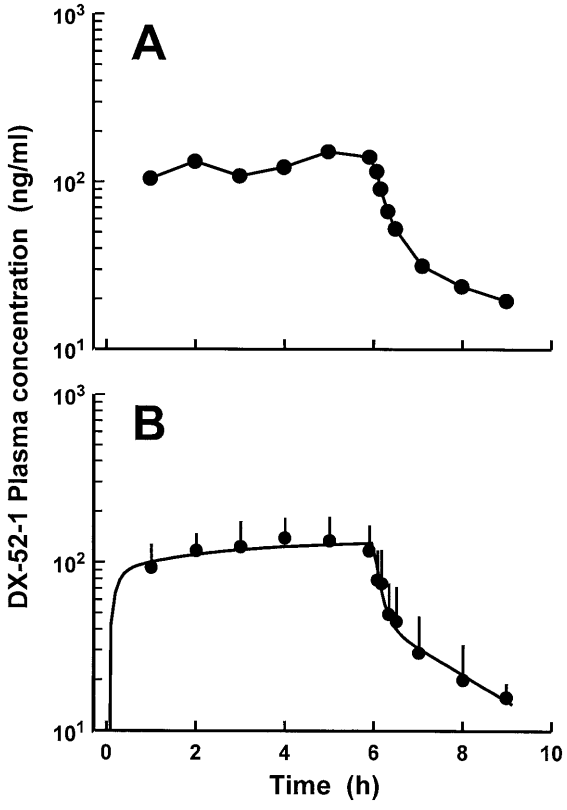


Fig. 2 **A** Representative plasma concentration-time profile of DX-52-1 for an individual patient treated with 6.0 mg/m^2 of the drug given as a 6-h continuous i.v. infusion. The observed DX-52-1 plasma concentrations are shown connected sequentially with line segments. **B** Mean plasma concentration-time curve constructed from all available data for the entire cohort of 19 patients evaluated at the 6.0 mg/m^2 dose level. The geometric means of the individual DX-52-1 plasma concentrations at each nominal sample time are shown and the vertical lines represent standard deviations. The solid line is the best-fit curve determined by nonlinear regression analysis of the mean profile

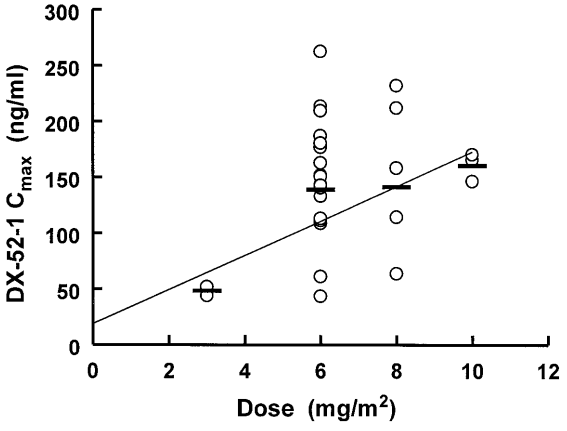


Fig. 3 Plot demonstrating the relationship between the C_{max} values of DX-52-1 achieved during the infusion and the administered dose. The points (○) represent the observed values in individual patients and the horizontal bars depict the geometric mean values obtained for each group. The solid line was generated from linear regression analysis of the mean values which afforded a correlation coefficient of 0.916

Table 4 Mean pharmacokinetic parameters of DX-52-1 determined at the 6.0 mg/m^2 dose level

Parameter	Noncompartmental analysis of individual plasma profiles[a]	Nonlinear regression analysis of the mean plasma profile[b]
C_{max} (ng/ml)	138.8 ± 59.3	129.8 ± 4.8
AUC (ng·h/ml)	807.9 ± 154.3	811.6 ± 30.0
CL (l/h/m^2)	7.43 ± 1.41	7.39 ± 0.27
$t_{1/2,1}$ (h)	ND	0.11 ± 0.03
$t_{1/2,z}$ (h)	1.52 ± 1.03	1.87 ± 0.32
MRT (h)	1.23 ± 0.46	1.11 ± 0.12
V_{ss} (l/m^2)	9.53 ± 2.98	8.20 ± 0.95

[a]Values are the geometric mean ± SD calculated from the individual parameter estimates for the second cohort of 12 patients evaluated at the 6.0 mg/m^2 dose level. The available data from the first group of patients treated at this dose level were included in calculations of the mean C_{max} ($n = 19$) and $t_{1/2,z}$ ($n = 16$)

[b]Estimated parameter ± SE determined from the best-fit of a biexponential equation to the mean plasma concentration-time curve prepared using all available data from a total of 19 patients treated with 6.0 mg/m^2 DX-52-1

improvement in the data was afforded by calculating the mean values of the observed DX-52-1 plasma concentrations at each time-point using all available data from 19 patients treated at the MTD. The resulting mean plasma concentration-time profile was very well described by the equation for zero-order drug input with biexponential disposition (Fig. 2B). Values of the pharmacokinetic parameters for DX-52-1 estimated by nonlinear regression analysis of the mean plasma profile are also given in Table 4 for comparison. These estimates were in excellent agreement with the mean values ascertained by noncompartmental analysis of the individual plasma profiles.

There was a very strong correlation ($r = 0.916$) between the observed C_{max} and estimated AUC values for the 17 patients studied at the 6.0 and 8.0 mg/m^2 dose levels with the more completely defined plasma profiles. This provided the justification to employ C_{max} as the pharmacokinetic variable for assessing the strength of correlations with the clinical data and for using C_{max} values from 13 of the initial patients entered into the study for whom the AUC could not be determined in these analyses. There were no significant correlations ($r < 0.4$) between any pretreatment serum chemistry value and the C_{max} of DX-52-1 observed in 19 of the 22 patients evaluated at the 6.0 mg/m^2 dose level. Furthermore, employing all available data sets from the entire cohort of patients ($n = 28$), no significant correlations were evident between C_{max} and the maximum percent change in any clinical laboratory variable ($r < 0.24$) during the first cycle of therapy. However, there was the suggestion of a moderate relationship between the weekly dose of DX-52-1 and the maximum percent decreases in serum potassium levels ($r = 0.61$), hemoglobin ($r = 0.55$), platelet count ($r = 0.68$) and hematocrit ($r = 0.59$).

Discussion

We conducted a phase I dose-escalation study of DX-52-1 which had not previously been administered to humans. The MTD was established as 6.0 mg/m^2 delivered by a 6-h i.v. infusion each week for 4 weeks followed by an interval of 2 weeks without drug. The DLTs were clinical gastrointestinal symptoms (severe abdominal pain, nausea, vomiting, diarrhea) and liver function abnormalities (hyperbilirubinemia, transaminitis, and elevated alkaline phosphatase). These toxicities were frequent and severe at doses only slightly above the MTD, despite aggressive supportive interventions with antiemetics and antimotility agents. Life-threatening toxicities occurred in two of three patients treated at 10 mg/m^2. Moderate toxicity was observed at the MTD in 12 of 35 courses. The episode of grade 3 elevation in creatinine was not secondary to acute tubular necrosis and resolved with discontinuation of therapy. Myelosuppression was mild.

No responses were seen in any of the 24 evaluable patients. A single patient treated at a dose greater than the MTD (10 mg/m^2) had stable disease at the first planned disease assessment (3 months), but had progressed by the second assessment (6 months). All other patients experienced progressive disease by the first tumor assessment after two cycles of therapy. These disappointing results may be at least partially explained by the heavily pretreated nature of our study population. Half of the patients had previously received three or more prior chemotherapeutic regimens.

Two bioanalytical methods for DX-52-1 based upon reversed-phase HPLC with UV detection were independently developed for preclinical pharmacokinetic studies [1, 3]. The lowest concentration of DX-52-1 that could be measured in plasma by either assay was in the 0.1–0.5 μg/ml range. With this level of sensitivity, the drug could only be monitored for about 30 min after treating mice by bolus i.v injection with doses of 12 or 75 mg/m^2. In contrast, the design of this initial phase I clinical trial of DX-52-1 involved the administration of a considerably lower starting dose of 3.0 mg/m^2 delivered as a more prolonged 6-h i.v. infusion. A substantial improvement in assay sensitivity was necessary to ensure that peak plasma concentrations of the drug achieved in patients could be measured, as well as to adequately define the decline in plasma levels following the end of the infusion.

Developing a more sensitive analytical method for DX-52-1 proved to be challenging [22]. The compound lacked structural features that could be readily exploited by the more sensitive conventional methods of detection for HPLC. The highly polar character of the drug presented difficulties in efficiently isolating it from plasma and separating it from endogenous interferences. The assay that was developed for this study, which was necessary to achieve the high selectivity required for low wavelength UV detection, involved extracting DX-52-1 in its zwitterionic form from plasma into an immiscible organic solvent and subjecting the extract to a fully automated two-dimensional separation using HPLC columns with different retention characteristics under isocratic reversed-phase conditions. The lowest concentration of the drug in plasma quantified with acceptable accuracy and precision by the method was 2.5 ng/ml. This proved to be sufficiently sensitive for defining the pharmacokinetics of DX-52-1 in cancer patients when given as a 6-h i.v. infusion.

A cohort of 22 patients was ultimately treated with the MTD of DX-52-1 and data amenable to the estimation of pharmacokinetic parameters by noncompartmental methods were obtained from 12 of these patients. Analysis of the mean plasma concentration-time profile for patients treated at the 6.0 mg/m^2 dose level indicated that the drug exhibited apparent biexponential disposition characterized by an initial phase with a 6.6-min half-life and a $t_{1/2,z}$ of 1.9 h. The magnitude of the MRT (1.23 ± 0.46 h) was concordant with the relatively rapid elimination of drug from plasma. Consistent with the hydrophilic nature of DX-52-1, which bears a full negative charge at physiological pH [22], the V_{ss} (9.5 ± 3.0 l/m^2) was less than 50% of the

volume of total body water (0.66 l/kg). This implies that the drug may be largely confined to the extravascular space [12]. Consideration of the small V_{ss} together with the relatively rapid CL suggests that the drug is probably not extensively bound to plasma proteins; however, this remains to be verified experimentally.

The range of doses evaluated during the course of this phase I study was relatively narrow, with the maximum administered dose of 10.0 mg/m^2 being only 3.3 times greater than the starting dose. Although the observed C_{max} tended to increase proportionately with escalations in the dose, the values displayed a relatively high degree of interpatient variability, which was particularly evident in the expanded cohort of patients evaluated at the 6.0 mg/m^2 MTD. The available data are not sufficient to conclusively establish whether DX-52-1 exhibits linear pharmacokinetic behavior.

There was no evidence of a significant relationship between C_{max} and the maximum change in any clinical laboratory parameter relative to the pretreatment value observed in patients during the first cycle of therapy. In contrast, the weekly dose of the drug was moderately correlated with the relative nadirs for the serum potassium level, platelet count, hemoglobin, and hematocrit. This disparity suggests that the toxicities resulting in these effects could be mediated by a metabolite rather than the parent drug. The highly specific nature of the assay used to determine plasma concentrations of DX-52-1 precluded the ability to detect any drug metabolites. However, a drug disposition study using ^{3}H-labeled DX-52-1 has indicated that the drug is rapidly converted to systemically circulating metabolites in mice and dogs [3]. The unchanged drug only accounted for a small fraction of the total radioactivity in plasma within 15 min of i.v. injection of the radiotracer dose.

It has been suggested that quinocarmycin resulting from in vivo formation of the oxazolidine ring and elimination of cyanide represents a potential metabolite of DX-52-1 [28]. The physiological significance of this transformation follows from confirmed observations that quinocarmycin can slowly generate reactive oxygen species through an auto-redox reaction, which could give rise to various nonspecific host toxicities, whereas DX-52-1 does not have this capacity [24, 27]. Although pH-dependent conversion of DX-52-1 to quinocarmycin in aqueous solution has been observed in our laboratories, as well as by others [22, 25], the compound has been found to be surprisingly stable when incubated in human plasma at 37°C in vitro [22]. To further examine this question, an indirect method was devised to employ our assay to measure quinocarmycin in plasma by quantitatively converting it to DX-52-1 by in situ hydrocyanation prior to isolation from the sample matrix for chromatographic analysis. An increase in the measured concentration of DX-52-1 upon reassaying samples with hydrocyanation relative to direct analysis of the sample would indicate the presence of quinocarmycin. Preliminary application of this technique on a limited number of plasma specimens acquired from patients near the end of the 6-h infusion of DX-52-1 failed to detect the presence of quinocarmycin.

One of the primary reasons for characterizing the pharmacokinetics of an investigational new anticancer agent in the context of a phase I trial is to assess whether a tolerable dose and schedule of the drug produces a potentially effective pattern of systemic exposure as indicated by preclinical activity studies. The only preclinical information previously reported pertains to two experiments in which mice were treated with 12 or 75 mg/m^2 doses of DX-52-1 by bolus i.v. injection [1, 3]. Peak plasma concentrations of the parent drug were in the range 7–10 μg/ml and the levels decayed rapidly, with an apparent half-life of 4–6 min, which is comparable to the $t_{1/2,1}$ observed in cancer patients.

Optimal efficacy against xenografts of tumors established from human melanoma cell lines, implanted subcutaneously in nude mice, has been observed with repeated administration of the drug by the intraperitoneal route [16]. Studies of the efficacy of DX-52-1 administered as a 6-h i.v. infusion against preclinical tumor models in vivo or its pharmacokinetics following intraperitoneal injection to mice have not been described. Further, the limited data on the i.v. pharmacokinetics of DX-52-1 in mice have no predictive value for the plasma profile associated with the intraperitoneal route of administration. This follows from the observation that i.v. doses of 50–100 mg/kg are acutely toxic [1], whereas tumor-bearing mice evidently tolerate repeated treatment with 60 or 90 mg/kg given intraperitoneally reasonably well [16]. This suggests that the drug is slowly absorbed from the peritoneal cavity into the bloodstream, resulting in a much lower peak plasma concentration as compared to rapid i.v. injection. Therefore, the mean plasma concentration-time curve of the drug in cancer patients treated with the MTD cannot be compared to plasma profiles corresponding to either the therapeutically optimal dosing regimen or clinical administration schedule in mice.

The mean C_{max} of DX-52-1 in the cohort of patients treated at the 6.0 mg/m^2 MTD (0.39 ± 0.17 μM) was more than 100 times lower than the average LC_{50} for all cell lines in the NCI in vitro anticancer screen (53 μM) and 28 times below the average LC_{50} for the nine cell lines comprising the melanoma subpanel (11 μM), which exhibited the greatest sensitivity to the growth inhibitory effects of DX-52-1[1]. The concentrations of drug eliciting an apparent cytostatic response in the screen, as indicated by total growth inhibition of the exposed cells, were not much lower, with average values of 1.5 μM for the melanoma cell lines and 7.4 μM for the entire panel. Thus, plasma levels of DX-52-1 achieved clinically during the 6-h infusion of the MTD were considerably lower than the concentrations required for cytostatic or cyto-

[1]Results for the evaluation of DX-52-1 in the NCI human tumor cell line screen were obtained by accessing public data provided by the Developmental Therapeutics Program, DCTD, NCI, at the following website: http://dtp.nci.nih.gov

toxic activity against sensitive human tumor cell lines in vitro with a 48-h exposure period. The concentration and exposure time associated with in vitro antiproliferative effects are not intrinsically predictive parameters for the pattern of systemic exposure that must be achieved in order for a drug to be clinically effective. However, the weekly dose intensity for one of the most efficacious treatment schedules identified during in vivo antitumor efficacy studies with DX-52-1 in mice (120 mg/kg, 360 mg/m^2) was 60 times greater than the 6.0 mg/m^2 maximum weekly dose tolerated by cancer patients. The administration of even moderately lower doses proved to be substantially less effective against the melanoma xenograft models. When considered together, these findings certainly evoke concern about the prospect for realizing clinical activity with DX-52-1.

In summary, DX-52-1 proved to be a poorly tolerated agent, producing severe and life-threatening hepatic and gastrointestinal toxicities. The investigated schedule failed to achieve drug levels adequate to inhibit either in vitro or in vivo tumor growth. No responses were observed in a cohort of 22 patients treated at the MTD. Given these findings, DX-52-1 does not appear to warrant further consideration for future phase II studies.

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