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ORIGINAL ARTICLE

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Toxicity of a quinocarmycin analog, DX-52-1, in rats and dogs in relation to clinical outcome

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Abstract *Purpose*: Quinocarmycin analog DX-52-1 is a cyanated derivative of quinocarmycin, a compound isolated from cultures of *Streptomyces melanovinaceus*. DX-52-1 was selected for preclinical development because it showed efficacy against melanoma cell lines in the NCI human tumor cell screen and melanoma xenografts in mice. This report describes studies in rats and dogs to determine the maximum tolerated dose (MTD) and identify dose-limiting toxicities (DLT) in each species in different regimens to establish a safe starting dose and potential target organs of DX-52-1 for phase I clinical trials. *Methods*: DX-52-1 was administered to Fischer 344 rats using repeated intravenous (i.v.) slow bolus injections following q3h×3 and q3h×3,q7d×3 regimens, and to beagle dogs using a single injection, 6-h continuous i.v. infusion (c.i.v.) and weekly 6-h c.i.v. for 3 weeks. Endpoints evaluated included clinical observations, body weights, hematology, serum clinical chemistry, and microscopic pathology of tissues. *Results*: The MTD of DX-52-1 was a total dose of 18 mg/m^2 body surface area for q3h×3 administration in rats and 30 mg/m^2 for a single c.i.v. administration in dogs. The total dose MTD for rats on a weekly (q3h×3,q7d×3) regimen was 54 mg/m^2, and for dogs on the weekly ×3 (6-h c.i.v.) infusion was 60 mg/m^2. In rats, significant elevations in blood urea nitrogen and creatinine were observed together with acute renal tubular necrosis histologically. Modest increases in liver enzymes were also observed, as were decreases in reticulocytes that were unaccompanied by histologic changes in liver and bone marrow. In dogs, adverse signs included vomiting/retching, diarrhea, and transient hypothermia; also red blood cells, hemoglobin, hematocrit, and lymphocytes were decreased. Histologic evaluation of tissues from dogs revealed necrosis and cellular depletion of the bone marrow, and extensive damage to the entire gastrointestinal tract, including marked cellular necrosis of the mucosa and lymphoid necrosis of the gastrointestinal associated lymphoid tissue. Destruction of the mucosal lining of the intestinal tract was likely responsible for dehydration, toxemia, septicemia, and shock seen in moribund dogs. *Conclusions*: The MTD values were comparable between rats and dogs given roughly similar dose regimens (single dose or weekly) and both species tolerated a higher total dose with weekly administration. However, the principal target organ responsible for DLT in rats was the kidney, whereas in dogs, the most severe effects were on the gastrointestinal tract and bone marrow. Both renal and gastrointestinal toxicities were reported in patients after 6-h c.i.v. infusions in a limited phase I clinical trial, indicating that neither animal model alone was predictive of DX-52-1-induced toxicity in humans, and that both species were required to define human toxicity.

Keywords Quinocarmycin analog DX-52-1 · Renal toxicity · Bone marrow toxicity · Gastrointestinal toxicity · *Streptomyces melanovinaceus* · Species differences

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Introduction

The quinocarmycin analog DX-52-1 (Fig. 1) is a cyanated and more stable derivative of quinocarmycin (KW2152), a compound isolated from cultures

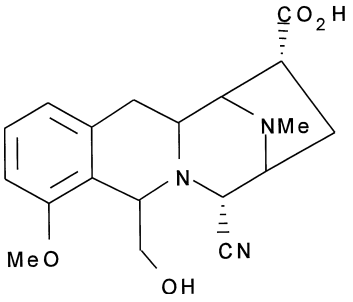

Fig. 1 Chemical structure of DX-52-1

of *Streptomyces melanovinaceus* [17, 18, 21]. Both compounds are structurally related to the mitomycin and saframycin families and have good antibiotic and antitumor activity [8, 11, 14, 15]. DX-52-1 and KW2152 have shown cytotoxic specificity for melanoma cell lines in the National Cancer Institute (NCI) human tumor cell line screen. Furthermore, these drugs have been shown to cause partial and complete regressions of subcutaneously implanted LOX IMVI and MEXF 989 melanoma xenografts [5, 16]. Antitumor activity has been found to be better after multiple doses than after a single dose and exceeds that of 11 of 12 standard clinical antitumor drugs [4, 16]. Earlier, KW2152 had received a limited clinical evaluation in Japan that did not include melanoma patients [12]; the trial was terminated because of toxicity following daily treatment for 14 days (Hirata, personal communication, as cited in reference 16).

DX-52-1 was chosen for further development by the NCI because of a lack of effective agents for the treatment of human melanoma and because its toxicologic profile might be different than that of KW2152, which contains an unstable oxazolidine ring [16]. Because of the lower toxicity that occurred in patients given KW2152 on a less-frequent regimen and the good preclinical antitumor activity observed for both agents with intermittent therapy, an interrupted schedule of drug administration was considered as a way to avoid unacceptable toxicity while retaining acceptable efficacy. The studies described here were conducted in two phases using a previously outlined approach [20]. In the preliminary toxicity phase, DX-52-1 was administered intravenously (i.v.) to rats and dogs to determine the maximum tolerated dose (MTD) and to identify the principal target organs of toxicity in each species. In the subsequent, IND-directed toxicity phase, definitive studies were performed in these species using various schedules to determine the dosing regimen that produced the least toxicity and might allow the drug to enter phase I clinical trials. Pharmacokinetic (PK) studies were also conducted for data on exposure levels at which the toxic effects of DX-52-1 occur. The results from the animal studies are compared and discussed in the context of current NCI test objectives and subsequent clinical trials of DX-52-1 to determine which animal model more accurately predicted the toxicities observed in patients.

Materials and methods

Animals

Rats

Fischer 344 rats (6–8 weeks old) were supplied by Charles River Laboratories (Hollister, Calif.) and quarantined for 4–7 days before study initiation. They were housed three or five per cage in polycarbonate cages with hardwood bedding in environmentally controlled rooms (a minimum ten air changes per hour, 20–22°C, 44–66% humidity, 12-h on/12-h off light cycle) with free access to rodent chow and UV-sterilized deionized water.

Dogs

Beagle dogs were obtained from Marshall Farms (North Rose, N.Y.) and Hazleton Research Products (Kalamazoo, Mich.) and were 7–17 months old at the start of each study. During quarantine (14 days), each dog was given a complete physical examination; all health parameters were normal. Dogs were housed individually in 4×8-ft enclosures with a minimum of ten air changes per hour, at 21–24°C, 30–70% humidity, and a light cycle of 12-h on/12-h off. All dogs had free access to untreated tap water and access to canine chow for 2 h daily.

Chemicals

DX-52-1 (NSC 607097) was obtained from the NCI Repository (Ogden Bioservices). Purity was 98% as determined by high-performance liquid chromatography (HPLC). DX-52-1 was dissolved and administered in 25 m*M* sodium phosphate buffer, pH 7.0. The dose solutions were confirmed by HPLC before administration to be within ±10% of the nominal concentrations. Stability under the conditions used in the infusion experiments were confirmed by removal of a dose solution sample from each bag at the end of the infusion and subsequent analysis by HPLC.

Study design

Preliminary single- and multiple-dose (five consecutive days) toxicity studies were performed in mice because this species had been used for earlier efficacy studies [16]. The mouse toxicity results (not included here) were used to estimate doses for subsequent rat and dog toxicity studies. Study designs for the rat and dog studies are summarized in Table 1. The experimental protocols were approved by SRI International's Institutional Animal Care and Use Committee (IACUC). For cytotoxic antitumor drugs, it is more accurate to compare toxicity findings at doses based on body surface area rather than body weight [7], although the latter units are also presented in Table 1 to facilitate comparison.

DX-52-1 was administered to rats as a slow injection (delivered over 60 s) via the lateral tail vein. Dogs received the compound either as a single i.v. bolus injection or as a c.i.v. for 1 h or 6 h via the jugular vein. In the multicycle studies, DX-52-1 was administered to the animals weekly. Dogs were tranquilized (0.04 mg/kg acepromazine hydrochloride and halothane) during implantation of catheters (V-Cath ML, 20-gauge single lumen catheter; HDC Corporation, San Jose, Calif.) on days 1 (day on which the first dose was administered) and 8; day-8 catheters were left in place for use on day 15.

Clinical and histopathologic evaluations

Blood was drawn from rats from the retro-orbital plexus under CO_2 anesthesia and from dogs via the cephalic vein at scheduled sampling times (Table 1). Blood samples for hematology were collected with EDTA, and white blood cell count (WBC), ery-

Table 1 Study designs of iv studies with DX-52–1

Species	No. and sex	Regimen	Dose level	Histopathology evaluation (days of scheduled death/ termination)	Clinical pathology (days evaluated)
Dog	1M/1F	Single i.v. injection	0, 18, 30, 42, 120 mg/m^2 (0, 0.9, 1.5, 2.1, 6 mg/kg)	None	−3, 1
	1M/1F	1 h c.i.v.	2, 10 mg/m^2 (0.1, 0.5 mg/kg)	None	−3, 3, 5, 8, 15
	1M/1F	6 h c.i.v.	2.5, 5 mg/m^2/h (0.125, 0.25 mg/kg/h)	15	−3, 1 (6, 12, 24 h after start of infusion), 5, 8, 11, 15, 22
	2M/2F[a]	6 h c.i.v., q7d×3 days 1, 8, 15)	0, 2, 3.34, 5, 7.5 mg/m^2/h (0, 0.10, 0.167, 0.25, 0.375 mg/kg/h)	16, 43	−10, −4, 1 (6, 12, 24 h after start of infusion), 5, 12, 19, 24, 28, 29, 36, 43
Rat	6M	q3h×3 slow i.v. injection	0, 3.6, 9, 22.2, 45, 90 mg/m^2/injection (0, 0.6, 1.5, 3.7, 7.5, 15 mg/kg/injection)	5, 15	SubgroupI: −4, 5; subgroup II: 2, 10, 15
	15M/15F (5M/5F per subgroup)	q3h,q7d×3 (days 1, 8, 15) slow i.v.injection	0, 3, 6, 9 mg/m^2/injection (0, 0.5, 1.0, 1.5 mg/kg/injection)	16, 43	Subgroup I: −4, 5, 16; subgroup II: 4, 9, 19, 29, 43; subgroup III: 2, 12, 24, 36, 43

[a]Except 1M/1F for the 3.34 and 7.5 mg/m^2/h dose levels

throcyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were measured with a Coulter STKR automated hematology analyzer. Differential leukocyte count (neutrophils, lymphocytes, eosinophils, basophils, and monocytes), platelet count, and reticulocyte count were determined microscopically with Wright/Giemsa- and methylene blue-stained peripheral blood smears. The following serum chemistry measurements were performed using a Boehringer Mannheim/Hitachi 736 or 717 analyzer: blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose, and creatinine. In addition, prothrombin time, total protein, creatine kinase (CK), lactate dehydrogenase (LDH), sodium, potassium, and chloride were analyzed in dogs only.

Necropsy with complete gross and microscopic examination was conducted on all organ systems of dogs and rats in the definitive studies and on animals killed in a moribund condition during the course of all the studies. Sections (5 μm) of paraffin-embedded tissues were prepared and stained with hematoxylin and eosin for microscopic evaluation.

Plasma drug determinations

Blood samples were collected from the tail vein at predetermined times from 2 to 120 min after dosing of either 90 or 150 mg/m^2 in a separate rat PK study (not listed in Table 1), and from the cephalic vein in dogs at intervals from 2 min to 24 h following a single bolus injection at 120 mg/m^2 in the preliminary toxicity study and from 1 to 7 h after the start of the 6-h infusion at 2.5 or 5 mg/m^2 per h. The samples were prepared for analysis by placing 100 μl plasma, 140 μl 50 m*M* phosphate buffer (pH 7.0), and 10 μl concentrated perchloric acid in a 0.5-ml microcentrifuge tube and vortexing at 16,000 *g* for 5 min. The resultant supernatant (200 μl) was subjected to HPLC analysis as follows: Waters 600 System chromatograph; Beckman Ultrasphere ODS (4.6×250 mm) column; 50 m*M* phosphate buffer, pH 4.36/acetonitrile (4:1) mobile phase, 1.0 ml/min flow rate; UV detection at 220 nm (Waters 991 photodiode array detector) and fluorescence detection at 272 nm excitation and 298 nm emission (Waters 470 fluorescence detector); Waters Millennium 2010 chromatography integrator (version 2.0); 50 μl injection volume (Waters 717 Plus autosampler). Retention times were approximately 6.60 min (UV) and 6.67 min (fluorescence). Plasma samples from the rat PK study and the dog bolus study were analyzed at SRI International using the Rstrip II exponential curve stripping and parameter estimation program, version 2.02 (MicroMath Scientific Software, Salt Lake City, Utah). Samples from the dog infusion study were analyzed in the laboratory of Dr. John McCormack, University of Vermont (Burlington, Vt.), using essentially the same method.

Statistical evaluation

Overall effects of dose on body weights, body weight gains, and clinical pathology data in rats were evaluated by one-way analysis of variance followed by Dunnett's test comparing each treated group with the respective control group (null hypothesis rejection, $P < 0.05$). In the dog studies, statistical evaluation was not performed because of the small numbers of animals; prestudy values for each individual served as the control values.

Results

Rats

Q3h×3

In the initial study, groups of 6 male rats were treated with three i.v. injections of DX-52-1 at 3-h intervals (q3h×3) and then evaluated for 2 weeks. This same schedule of administration had been used previously in mouse xenograft studies and shown to produce antitumor activity (NCI, unpublished results). All the rats that received doses ≥22.2 mg/m^2 died within 24 h. In the 9 mg/m^2 group, one rat was found dead on day 6; no necropsy was performed as the tissues were severely autolyzed. On day 5, animals in the 9 mg/m^2 group had an 18% decrease in body weight compared with the control group. By day 10, significant changes in RBC, HGB, and HCT (decreased 17%) and reticulocytes (increased 1.5-fold relative to control values) were observed; however, body weight had recovered. On day 15, there was a significant elevation in MCV and reticulocytes were still significantly elevated, indicating a

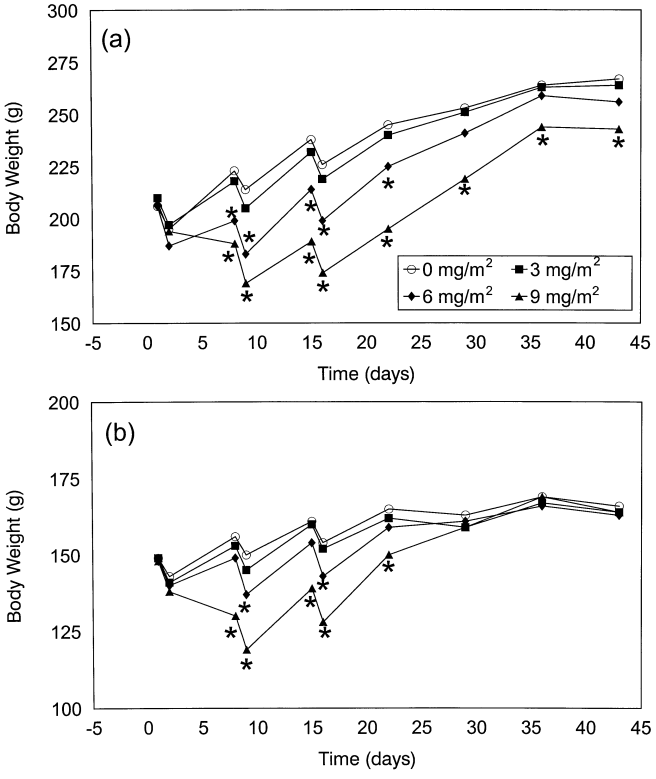

Fig. 2a, b Effect of DX-52-1 administered to rats as three i.v. injections given at 3-h intervals once weekly for 3 weeks (q3hx3,q7dx3) on mean body weights in male (**a**) and female (**b**) rats (*$P < 0.01$)

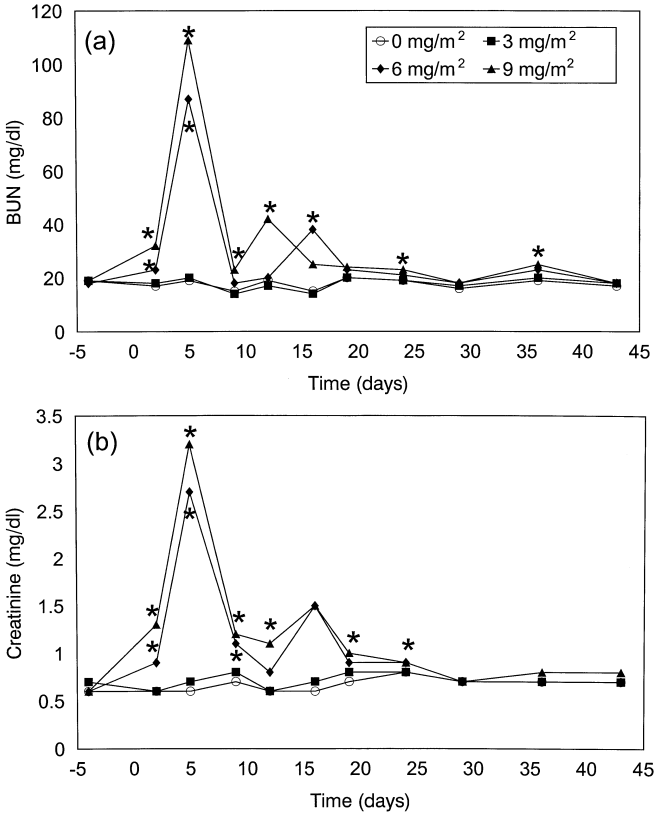

Fig. 3 Effect of DX-52-1 administration to male rats as three i.v. injections given at 3-h intervals once weekly for 3 weeks (q3hx3,q7dx3) on mean BUN (**a**) and serum creatinine (**b**) levels (*$P < 0.01$)

compensatory response to the mild anemia. No effects were seen on clinical chemistry parameters throughout the study. No hematologic effects were observed at the lowest dose level tested (3.6 mg/m^2).

Q3h×3, q7d×3

In the definitive study, 15 rats of each sex per dose group received three i.v. injections of DX-52-1 at 3-h intervals (3, 6, or 9 mg/m^2) once a week for 3 weeks (days 1, 8, and 15). The rats were killed on either day 16 or day 43 and underwent gross and microscopic examination. One female rat in the 9 mg/m^2 group was found dead on day 10. Dose-related adverse signs (hunched posture, rough coat, emaciation, hypoactivity, diarrhea, chromodachryorrhea) were observed in all DX-52-1 dose groups. Body weights were significantly lower in the 6 and 9 mg/m^2 groups, with maximal decreases on day 9 of 15% and 21% in males (Fig. 2a) and 9% and 21% in females (Fig. 2b), respectively.

Several hematologic parameters were altered following DX-52-1 administration. RBC, HGB, HCT, and reticulocyte levels were decreased in a dose-related and time-dependent manner. The differences were statistically significant at the higher dose levels. At the nadir on day 16, RBC count, HGB, and HCT were decreased 13% and 21–23% in males in the 6 and 9 mg/m^2 groups, respectively, and 13–16% in females in the 9 mg/m^2 group only. By day 43, the RBC count in male rats in the high-dose group remained 12% lower than control values. Reticulocyte counts were 81–94% lower in the 9 mg/m^2 group on day 9, but by days 12 and 16, reticulocytes were elevated three- to fourfold relative to the control groups and remained so until day 43. These changes were accompanied by significant elevations in MCV and MCH in the medium- and high-dose male groups and in the high-dose female group on day 24 that persisted to study termination. These effects were considered indicative of a compensatory response to the anemia previously seen in these rats. Modest, stress-related increases in neutrophils were also observed occasionally in the 6 and/or 9 mg/m^2 groups.

Rats in the 6 and 9 mg/m^2 groups showed substantial increases in BUN (Fig. 3a) and creatinine (Fig. 3b), with peak levels occurring on day 5 that were four- to sixfold higher in males and two- to fourfold higher in females relative to the control group values. ALP, AST and ALT were also modestly increased (up to threefold); these changes were transient and not evident by the third injection.

Histopathologic evaluation of tissues on day 16 revealed multifocal and diffuse acute tubular necrosis of the renal cortex in all dose groups (minimal severity in

the 3 mg/m^2 group, males only, and mild to marked severity in both sexes in the higher dose groups). In addition, multifocal and diffuse postnecrotic tubular regeneration was observed at all three dose levels. Evidence of tubular regeneration was seen in some rats on day 43 also, but with diminished frequency and severity. No evidence of liver injury was seen in rats that survived to study termination.

Dogs

Preliminary studies

The single-dose toxicity of DX-52-1 in beagle dogs was initially assessed as an i.v. bolus administration. Doses of ≥18 mg/m^2 produced moribundity and death within 24 h. The dogs exhibited multiple toxic signs and organ damage, most notably in the small and large intestine, bone marrow and lymphoid tissues. Renal tubular necrosis was not seen except at the highest dose tested (120 mg/m^2). Subsequently, DX-52-1 was administered as a 1-h c.i.v. infusion at doses of 2 or 10 mg/m^2 per h. All dogs (two per dose group) survived, and clinical signs were limited to transient diarrhea and vomiting on the day of administration. A mild decrease in lymphocyte count on day 3 in the 10 mg/m^2 group was considered to be secondary to shock. There were no effects on clinical chemistry parameters.

6-h c.i.v.

Groups of two dogs each were given DX-52-1 as a 6-h c.i.v. infusion at either 2.5 or 5 mg/m^2 per h (total dose of 15 or 30 mg/m^2, respectively) to determine whether a larger total dose could be tolerated when the drug was administered more slowly. All four dogs vomited and had diarrhea with discolored stool on the day of treatment. A borderline hemolytic anemia was observed in three of the four dogs; in the male from the 5 mg/m^2 per h group, this condition appeared to persist to the end of the study (day 22). Stress-related transient changes in lymphocyte counts (77–94% decrease) and in neutrophil counts (71–192% increase) occurred within 24 h of starting the infusion. No clinical chemistry changes occurred that were attributable to the DX-52-1, and a histopathologic evaluation was not performed on these animals.

Weekly 6-h c.i.v. (q7d×3)

In the definitive toxicity study, DX-52-1 was infused for 6 h at various dose levels (between 2 and 7.5 mg/m^2 per h) on days 1, 8, and 15, followed by a 4-week recovery period. Both dogs at the highest dose level died within 24 h of starting the first infusion (7.5 mg/m^2 per h, total dose 45 mg/m^2). At the 5 mg/m^2 per h dose level, one dog became moribund and was killed within 24 h after completing the first infusion, two died after the second infusion, and the remaining dog after the third infusion. No dogs at the lower dose levels died during the study. Multiple toxic signs were seen in dogs that died early including vomiting and salivation, marked hypoactivity/prostration, dyspnea, and pale gums and/or mucous membranes. Rectal temperatures were transiently lowered (1–3°F) at the end of each infusion.

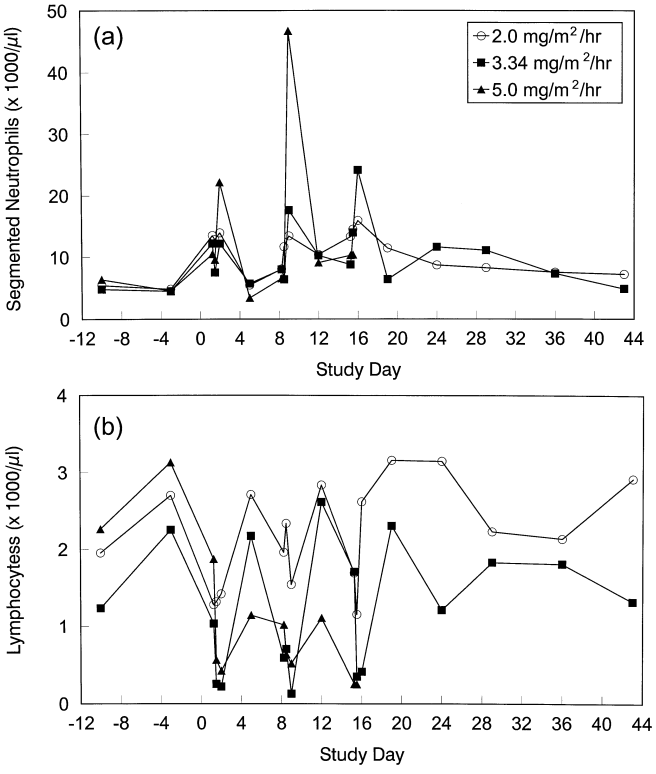

Fig. 4a, b Effect of three weekly 6-h continuous i.v. (c.i.v.) infusions of DX-52-1 on mean WBC differential counts in dogs. Results for male and female dogs were very similar and are combined (**a** segmented neutrophils, **b** lymphocytes)

Dogs temporarily lost body weight during the study. Maximal changes were evident on day 16 and averaged 8%, 13%, 18%, and 24% lower in the control, 2, 3.34, and 5 mg/m^2 per h dose groups, respectively, compared with pretreatment weights. Food intake was depressed following each infusion but had recovered to normal by the next infusion.

Dogs in the 5 mg/m^2 per h group had increased neutrophil counts (both mature and immature; Fig. 4a) with concurrently lower lymphocyte counts (decreased 70–90%) following the first infusion (Fig. 4b). Surviving dogs exhibited lymphocytopenia, but not always neutrophilia, following subsequent infusions. Both dogs in the 7.5 mg/m^2 per h group had similar leukocytic effects. RBC (Fig. 5), HGB, and HCT values were increased at 12 and/or 24 h after each infusion in the dogs receiving 5 and 7.5 mg/m^2 per h due to hemoconcentration. Nucleated RBCs were also observed in serum from several of these dogs.

In the sublethal dose groups (2 and 3.34 mg/m^2 per h), lymphocytopenia and/or neutrophilia were observed

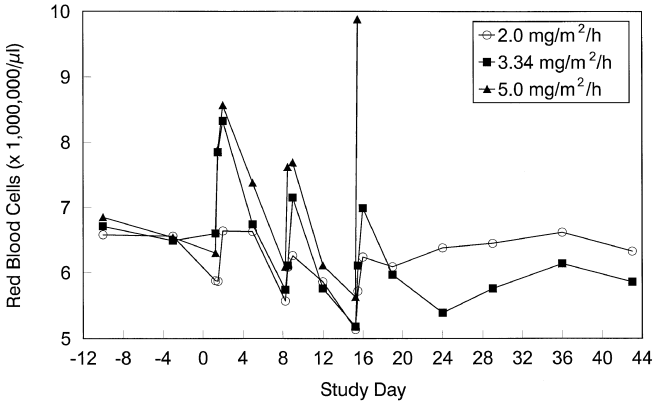

Fig. 5 Effect of three weekly 6-h infusion of DX-52-1 on mean RBC counts in dogs. Results for male and female dogs were very similar and are combined

following each infusion (6-h time point); however, lymphocyte and neutrophil counts returned to baseline levels within 2 days and remained at those levels during the recovery period (Fig. 4). Dogs in these groups also exhibited transient decreases (17–27%) in RBC (Fig. 5), HGB, and HCT occurring primarily on the days of the second and third infusions.

Clinical chemistry changes were observed at all dose levels. The incidence and severity of the changes during the 24-h period following each infusion were dose-related (data not shown). Marked elevations in LDH, ALT, AST, and CK at lethal dose levels were probably secondary to other events such as toxemia and shock. Elevations in BUN and creatinine were attributed to prerenal azotemia secondary to dehydration and vascular collapse or shock. Mild elevations in total protein and decreases in serum electrolytes and glucose accompanied these changes. At the lower, nonlethal dose levels modest elevations in ALP (2- to 3.5-fold), AST (3-fold), and ALT (4-fold) relative to baseline (pretest) values were occasionally seen; these changes were not cumulative and reversed after termination of the dosing regimen.

Histologic evaluation of dogs in the 5 and 7.5 mg/m^2 per h groups revealed extensive damage to the entire gastrointestinal (GI) tract comprising marked cellular necrosis of the mucosa and marked lymphoid necrosis of the GI associated lymphoid tissue (GALT), along with congestion and moderate hemorrhage, mild to moderate bone marrow necrosis and moderate to marked cellular depletion, lymphoid depletion and/or cellular necrosis of the spleen, thymus, lymph nodes, and tonsils. In the 2 mg/m^2/ per h group, moderate suppurative inflammation of the GALT (male dog only) and tonsils (both male and female dogs) were also observed on day 16. Mild splenic lymphoid depletion was observed in the male dogs from the 2 and 3.34 mg/m^2 per h groups but not in the females. These were generally less severe than at the higher dose levels and were considered reversible. No evidence of liver or kidney necrosis was observed in tissues from male or female dogs.

Table 2 Pharmacokinetic parameters from bolus i.v. administration of DX-52–1 to dogs and rats

Parameter	Dog[a]	Rat	
	120 mg/m^2	90 mg/m^2	150 mg/m^2
$t_{1/2}\alpha$ (min)	5.70	0.98	4.13
$t_{1/2}\beta$ (min)		7.26	17.42
$t_{1/2}\gamma$ (min)		22.65	36.77
Cp_0 (µg/ml)	25.80	129.81	121.49
$AUC_{0-\infty}$ (µg min/ml)	200.8	781.04	1631.20
MRT (min)	8.20	16.55	29.52
V_d (l/kg)[b]	0.25	0.63	0.81
Cl (ml/min/kg)[c]	29.88	19.21	15.33

[a]Values are an approximation only, based on three or four time points
[b]Volume of distribution (V_d) = Cl/k
[c]Clearance rate (Cl) = dose (µg/kg)/AUC

Pharmacokinetics

The results of the analysis of plasma samples from the dogs and rats receiving a single bolus dose of DX-52-1 are summarized in Table 2. DX-52-1 was detectable only at lethal doses in rats and dogs that received a single bolus injection. No measurable levels of DX-52-1 were found in any samples analyzed from the c.i.v. studies, due to insufficient sensitivity of the analytical method.

In the rat PK study, plasma levels of DX-52-1 decreased rapidly following doses of 90 or 150 mg/m^2. In this species, the data best fit an open three-compartment model. The volume of distribution (0.63 to 0.81 l/kg) indicated that the drug was widely distributed and that essentially no tissue binding occurred with DX-52-1. For the 150 mg/m^2 dose, the area under the curve was larger, clearance was lower, and the mean residence time was substantially longer than expected based on the PK results for the 90 mg/m^2 dose. These findings suggest that elimination processes were becoming saturated at the higher dose.

In both dogs that received a single bolus of 120 mg/m^2, DX-52-1 was detectable in the plasma for only 10 or 20 min after administration. The data fit an open one-compartment model, and the calculated parameters indicated rapid clearance of the drug and extracellular distribution (V_D < 0.7 l/kg). These values are approximations because they were based on only three or four time points. At 30 mg/m^2, the plasma concentration of DX-52-1 was 4.7 µg/ml at 2 min and was no longer detectable at 5 min.

Discussion

The principal aims of the preclinical toxicology program for anticancer drug development at the NCI are to establish a safe starting dose and dose escalation scheme to reach an MTD efficiently for maximal patient benefit and to define the dose-limiting toxicity

(DLT) (potential target organs) to assist in patient monitoring during phase I clinical trials. Anticancer drugs tend to be among the most toxic agents administered to humans and patients in early clinical trials are typically very ill. Consequently, aggressive dose escalation schemes have emerged in clinical practice and success often means selecting a high enough starting dose to minimize the number of escalations required to reach either the MTD or a biologically effective dose in humans. To optimize chances for clinical activity and safety, current NCI practice applies the concept of agent-directed studies within a pharmacologically guided framework to reliably extrapolate toxic effects across species for adequate design of clinical protocols with the best chance of activity [19]. In contrast to European practice in which only the rodent is used with emphasis on the mouse, the NCI conducts toxicity studies in two species, usually rat and dog, and has found the latter to be more precise for predicting MTD and DLT in humans in a limited data set [19]. The work reported here not only provides data on the mammalian toxicity of DX-52-1, but also expands the preclinical database and improves the extrapolation for predicting outcome in clinical trials of antineoplastic agents by correctly identifying all potential target organs.

The most notable observation in the present studies is that DX-52-1-induced effects were less extensive in the rat than in the dog and differed in the primary target organ. The elevations in BUN (Fig. 3a) and creatinine (Fig. 3b) that peaked on day 5 and diminished to control levels thereafter were indicative of a direct cytotoxic effect of DX-52-1 on the rat kidney and adaptation to the injury. These effects were confirmed histologically to be due to acute tubular necrosis and regeneration. Modest increases in AST, ALT, and ALP were observed, but these changes were probably secondary to the metabolic effects of azotemia on other tissues or to direct cytotoxic injury to hepatocellular and/or cardiac muscle that was not detectable morphologically. Likewise, the time-course changes in reticulocyte counts were interpreted as an early, but mild, bone marrow toxicity (not observed histologically) that was followed by a compensatory response to the anemia evident later (days 24 and 29). The DLT was therefore considered to be acute renal tubular necrosis.

In contrast to the rat, DX-52-1 produced extensive toxic effects in dogs, and the toxicity was qualitatively similar regardless of the schedule of administration. The most severe effects were in the GI tract and bone marrow with the GI toxicity being most immediately life-threatening. Destruction of the mucosal lining of the intestinal tract resulted in dehydration, toxemia, septicemia, and shock to the animals and the majority of clinical signs observed. A marked suppurative inflammation was also observed in the GALT of two dogs from the 5 mg/m^2 per h group; this was considered an early lesion premonitory to the lymphoid necrosis of the GALT. Other lesions included depletion of various lymphoid tissues and pancreatic acinar cell degeneration. The latter effect was mild at lethal doses of DX-52-1 and therefore considered of minimal importance relative to the other effects. Likewise, dose-related increases in ALT, AST, and/or ALP were observed; these changes were mild at nonlethal dose levels and not dose-limiting. Dogs given a single bolus injection of DX-52-1 exhibited tubular necrosis only at a dose level (120 mg/m^2) much higher than the minimal lethal dose (18 mg/m^2) in that species. Consequently, the dog alone as an animal model at nonlethal dose levels would not have predicted the kidney as a potential target organ in clinical studies.

Hematologic effects due to DX-52-1 treatment were also documented in rats and dogs. Periodic and transient decreases in RBC count, HGB, and HCT were observed at nonlethal doses of DX-52-1 and were ascribed to mild hemolytic anemia due to peripheral cytotoxicity rather than to toxicity in the bone marrow, since bone marrow toxicity was not observed at the lower doses. At higher (lethal) doses in dogs, the anemia was obscured by severe hemoconcentration, which most likely occurred secondarily to the severe GI toxicity. The presence of nucleated RBCs in the peripheral blood of these same dogs suggests at this dose level the occurrence of mild bone marrow toxicity and/or hyperadrenocorticism due to stress resulting from the moribund status of the animals; the substantial myelotoxicity in the dogs lends credence to the former interpretation. The leukocytopenia (usually marked lymphocytopenia) observed in some dogs prior to death (and in dogs at the lower dose levels) may also stem from direct cytotoxic effects on the bone marrow, but this effect invariably reversed before the next infusion and was not considered life-threatening.

DX-52-1 has limited structural similarity to the mitomycins and saframycins. Most compounds in these families produce dose-limiting myelotoxicity (regardless of schedule of administration), which is different from the toxicity profile for DX-52-1 [6, 10, 13]. One exception to this is BMY-25282, a mitomycin C analog. BMY-25282 administered i.v. to rats as a single dose or five daily doses produces acute toxicities (GI epithelial necrosis, myelotoxicity, and splenic lymphoid depletion) as well as delayed and irreversible cardiac, pulmonary, and renal toxicities [2]. However, weekly administration of BMY-25282 at the same total dose only produces tubular degeneration and slight glomerulopathy, which is similar to the effects seen with DX-52-1.

Factors that may be responsible for the species-specific toxicity are differences in the rate of clearance, principal route of excretion, and dwelling time in the respective target organ of each species, as well as inherent differences in tissue sensitivity to the drug. Studies of the kinetics of clearance (in mice and dogs) and disposition (in mice only) of tritium-labeled DX-52-1 after an i.v. injection demonstrated high radioactivity levels (parent compound and metabolites) in the gall bladder, kidney, liver, and lung at 15 min after admin-

istration of the compound to mice. Substantial (though unquantified) radioactivity was also present in the GI lumen and lesser amounts in other tissues, including bone marrow [9]. At 4 h, high levels of radioactivity were still present in the gall bladder, renal cortex, and GI lumen; however, by 24 h, minimal radioactivity was detectable in the GI lumen only. Approximately 62–68% of DX-52-1 was excreted in the urine and 24–28% in the feces in both species. In a different study, the plasma half-life of DX-52-1 in mice was 4–6 min [1] and urinary excretion contributed significantly to the clearance of DX-52-1, as evidenced by very high concentrations of the intact compound in urine (micromolar range) following both i.v. and nonparenteral administration (15–300 mg/m^2 dose; NCI, unpublished results). If clearance of DX-52-1 is similar in mice and rats, then it is reasonable to assume that high concentrations of urinary DX-52-1 could lead to renal toxicity, as was observed in rats. Unfortunately, precise clearance could not be determined in rats and dogs because of insufficient sensitivity of the HPLC method, and so the difference in target organ toxicity in these species cannot be explained pharmacodynamically based on the limited results in the present study.

To determine the maximum safe dose that could be administered, DX-52-1 was studied in dogs using various schedules of administration: a single iv bolus injection, a 1-h infusion, a 6-h infusion, and weekly 6-h infusions. Doses ≥18 mg/m^2 were lethal when DX-52-1 was administered as a single injection. When administered as a 6-h infusion, a weekly dose of 20 mg/m^2 (total dose 60 mg/m^2) was toxic but not lethal to the dogs. Therefore, dogs were able to tolerate a larger total dose of DX-52-1 when the duration of administration was lengthened and the drug was given intermittently. These results were anticipated based on those obtained earlier in mice with KW2152 [16].

The MTD of the quinocarmycin analog DX-52-1 is comparable between rats and dogs even though the schedule of administration was different for the two species (Table 3). For rats that received DX-52-1 as three slow i.v. injections at 3-h intervals (approximating a 6-h infusion), the MTD was ≥27 mg/m^2 (total dose). For dogs that received a 6-h infusion, the MTD was 30 mg/m^2. Moreover, the MTD was 54 mg/m^2 (total dose) for rats receiving three injections once a week for 3 weeks (q3h×3,q7d×3) and 60 mg/m^2 (total dose) for dogs receiving a 6-h c.i.v. infusion weekly for 3 weeks.

Based on the results from the rat and dog toxicity studies, the clinical starting dose recommended for a phase I clinical trial was 333 μg/m^2 per h or a total dose of 6 mg/m^2 which is one-tenth of the dog MTD. The clinicians chose to be somewhat more conservative and the phase I trial for DX-52-1 started with a dose of 3 mg/m^2 or one-twentieth of the dog MTD. Two phase I clinical trials were initiated with DX-52-1; however, both trials have since been terminated due to inability to achieve plasma concentrations that inhibited tumor growth in vitro or in vivo and ineffectiveness at the plasma drug concentrations that could be safely achieved [3]. From the limited results available, GI toxicity (abdominal pain, nausea, vomiting), hepatic toxicity (elevated ALP, AST, ALT, hyperbilirubinemia), renal toxicity (elevated serum creatinine, proteinuria, glucosuria, and hematuria) and myelosuppression (thrombocytopenia, lymphopenia) were most prominent; mild anemia was also noted. The DLTs were ascribed to clinical GI symptoms and liver function abnormalities. The estimated MTD was 6 mg/m^2 given as four weekly 6-h c.i.v. infusions (24 mg/m^2 total dose), which is approximately two- to threefold lower than the MTD in rats and dogs and considerably lower than the minimally effective dose in the mouse (about 100 mg/m^2 total dose; NCI, unpublished results).

Table 3 MTD values for DX-52–1

Species	Study	Dose level (mg/m^2 or mg/m^2/h or mg/m^2/injection)	Total dose	
			mg/m^2	mg/kg
Dog	1 injection	< 18	< 18	< 0.9
	1 h c.i.v.	> 10	> 10	> 0.5
	6 h c.i.v.	≤ 5	≤ 30	1.5
	6 h c.i.v., q7d×3	3.34	60	3.07
Rat	q3h×3	≤ 9	≤ 27	≤ 4.5
	q3h×3,q7d×3	6	54	9

In summary, the DLT of DX-52-1 given as multiple i.v. injections in rats was renal toxicity, whereas GI toxicity and myelotoxicity (lymphocytopenia) were dose-limiting in dogs following weekly 6-h c.i.v. infusions. Liver function changes and anemia are common to rats, dogs, and humans. The observation of both GI and renal toxicities in patients following 6-h c.i.v. infusions indicates that neither animal model alone in the protocols used was predictive of DX-52-1-induced toxicity in humans, and supports the view that testing in both species was required to define human toxicity. The dog model in these protocols predicted more toxic manifestations of DX-52-1 in the clinical setting than the rat, including the DLT, which is in accord with results from other retrospective studies [20].

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