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Toxicity of dolastatin 10 in mice, rats and dogs and its clinical relevance

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Abstract *Purpose*: Dolastatin 10 (DOL 10), an oligopeptide isolated from the sea hare *Dolabella auricularia*, has been shown to be a highly potent cytotoxic agent in a variety of human tumor cell lines. The purpose of this study was to conduct preclinical toxicity evaluations to determine the target organ(s) of toxicity and its reversibility, the dose-limiting toxicity and the maximum tolerated dose (MTD), and to use this information for arriving at a safe starting dose and dose schedule for phase I clinical trails. *Methods*: DOL10 was administered as a single intravenous bolus dose to CD2F1 mice, Fischer-344 rats and beagle dogs. Endpoints evaluated included clinical observations, body weights, hematology, serum clinical chemistry, and microscopic pathology of tissues. *Results*: The MTD (i.e. the highest dose that did not cause lethality but produced substantial toxicity) was approximately 1350 μg/m^2 body surface area (450 μg/kg) in mice, 450 μg/m^2 (75 μg/kg) in rats and ≤400 μg/m^2 (≤20 μg/kg) in dogs. Adverse signs were observed at doses ≥1350 μg/m^2 in mice, ≥150 μg/m^2 in rats and ≥400 μg/m^2 in dogs. Decreased weight gain or actual weight loss was observed at doses ≥1350 μg/m^2 in mice, ≥600 μg/m^2 in rats and ≥450 μg/m^2 in dogs. In all three species, the primary target organ of toxicity was the bone marrow, as indicated by decreases in the numbers of erythroid cells, myeloid cells, and megakaryocytes in the femoral bone marrow and by decreased white blood cell (WBC) and reticulocyte counts in peripheral blood. Marked neutropenia (i.e. >50% decrease compared to control animal or baseline values) was the principal effect on WBCs and occurred within a week of dosing. A mild anemia was evident 1 week after administering the drug to rats and dogs. The hematologic effects were transient and reversed by study termination. Other lesions at the MTD levels were cellular depletion and necrosis in lymphoid organs (rats and dogs), marked depletion of extramedullary hematopoietic cellular elements in the spleen (rats), thymic atrophy (mice and dogs), and minimal cellular necrosis in the ileum (rats). More extensive and severe pathology was observed in animals sacrificed in a moribund condition or found dead. *Conclusions*: Myelotoxicity was dose-limiting in all three species with mice being the least sensitive. In a phase I clinical trial, granulocytopenia was dose-limiting. Moreover, the MTD of DOL10 for rats and dogs is comparable to the human MTD. Therefore, the results from the preclinical toxicology studies correctly predicted a safe starting dose, the dose-limiting toxicity, and the MTD in humans.

Key words Preclinical toxicology · Cancer chemotherapy · Myelotoxicity · Dolastatin 10

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Introduction

Dolastatin 10 (DOL 10) is a linear peptide isolated from the sea hare *Dolabella auricularia* and consists of four amino acids (three of them unique to *D. auricularia*) linked to a complex primary amine at the carboxyl terminus [2, 17]. It is the most potent antineoplastic agent among the dolastatins, exhibits good activity in the National Cancer Institute's (NCI) intraperitoneal implanted P388 leukemia model, and is a highly potent antiproliferative agent in a variety of tumor cell lines [1, 5, 12].

In the NCI human tumor cell line screen (60 cell lines), the cell lines that are most sensitive to the cytotoxic effects of DOL10 are those that are typically inhibited by tubulin-binding agents. The mechanism of action of DOL10 has been extensively studied and the compound has been identified as an antimitotic agent

that prevents the polymerization of tubulin and consequently inhibits the synthesis of microtubules. Compared to other drugs that bind the vinca domain, DOL10 inhibits tubulin polymerization at a potency similar to that of phomopsin A and vinblastine and at a potency greater than that of maytansine (threefold lower) and rhizoxin (fivefold lower) [2]. In addition, DOL10 is a noncompetitive inhibitor of ^{3}H-vincristine binding to tubulin (apparent K_i = 1.4 μM) and is more potent than other vinca domain binding drugs [3]. DOL10 is also a potent inhibitor of tubulin-dependent GTP hydrolysis [2] and nucleotide exchange [3].

In mouse xenograft studies conducted by the NCI, intravenously administered DOL10 produced growth delays and complete regressions of subcutaneously implanted tumors (early stage LOX melanoma and advanced stage NCI-H522 non-small-cell lung carcinoma). DOL10 effectively inhibited tumor growth when given as a single i.v. injection, but did not exhibit activity when the same total dose was administered as multiple i.v. injections using various dosing schedules. This observation was attributed to the rapid liver metabolism of DOL10 [14]; possibly a lower dose given multiple times did not allow the drug to reach a ''threshold'' concentration necessary for antitumor activity. DOL10 has been shown to inhibit growth of hematopoietic progenitor cells [10]; therefore, it was expected that myelotoxicity would be a significant and possibly dose-limiting toxicity in animals and humans.

Based on the promising results from efficacy studies, preclinical toxicity testing of DOL10 was conducted in mice, rats, and dogs. The objectives of these studies were to determine the target organ(s) of toxicity and its reversibility, the dose-limiting toxicity and the maximum tolerated dose (MTD) following a single i.v. bolus dose. Further objectives were to use data from these studies for recommending an initial starting dose and schedule of administration for phase I clinical trials and to evaluate whether the preclinical toxicology studies were predictive of clinical trial responses [20].

Materials and methods

Animals

Rodents

CD2F1 mice (male only) were supplied by Frederick Cancer Research and Development Center (Frederick, Md.). Fischer-344 rats (male and female) were supplied by Charles River Laboratories (Kingston, N.Y.). The animals were 5–7 weeks old at receipt and were housed five per cage in suspended polycarbonate cages with Sani-Chips hardwood bedding at 18–24 °C and in a relative humidity of 30–67%. The light cycle was automatically controlled, 12 h on and 12 h off. All rodents had free access to purified tapwater via automatic watering and to Purina Certified Rodent Chow #5002.

Dogs

Beagle dogs were obtained from Hazleton Research Products, Inc. (Kalamazoo, Mich) and were 7–11 months old at study initiation. Dogs were housed individually in 4 × 8 ft wire mesh enclosures with a concrete floor and maintained at a temperature of 20–31 °C and at a humidity range of 40–70%. The light cycle was controlled automatically, 12 h on and 12 h off. All dogs had free access to tapwater via an automatic watering system and were fed Purina Certified Canine Chow #5007 once per day for 2 h.

Chemicals

DOL10 (NSC-376128) was obtained from the Developmental Therapeutics Program of the NCI. DOL10 formulations were prepared by dissolving the compound in 0.1 *M* sodium phosphate buffer, pH 7.1 (vehicle), and concentrations were confirmed to be within ±10% of target by high-pressure liquid chromatography (HPLC).

Study design

The designs of the various toxicology studies are outlined in Table 1. The experimental protocols were approved by SRI International's Institutional Animal Care and Use Committee. A preliminary toxicity study was performed in mice (600, 900, 1350, and 2025 μg/m^2) because this species had been used for earlier efficacy studies at the NCI. The data from this study were then used to select doses for the toxicity studies in rats (150, 450, 600, 1350, and 2025 μg/m^2). Separate dose range-finding and definitive toxicity studies were conducted in dogs (doses were 450 and 900 μg/m) in the first study and 200 and 400 μg/m^2 in the second study), but the results will be presented and discussed as though all the data were obtained from one study.

Dose solutions were administered as a single i.v. bolus injection via the cephalic vein in dogs and the lateral tail vein in rats and mice. Study day 1 was defined as the day DOL10 was administered to the animals. Animals were observed daily for adverse signs, and body weights, hematology and clinical chemistry measurements were performed on specified days throughout the studies as shown in Table 1. Histopathology evaluation was performed on animals sacrificed as scheduled or sacrificed due to moribundity (Table 1).

Clinical and histopathologic evaluations

Blood was drawn from mice and rats (not fasted) from the retro-orbital sinus under CO_2 anesthesia and from dogs (after overnight fasting) via the jugular vein at the sampling times indicated in Table 1. Blood samples for hematology were collected with EDTA. Erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin were measured with a Coulter STKR Automated Hematology Analyzer. Differential leukocyte count (neutrophils, lymphocytes, eosinophils, basophils, monocytes), platelet count, and reticulocyte count were determined microscopically from 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. Prothrombin time, total protein, creatine kinase, lactate dehydrogenase, sodium, potassium, and chloride were analyzed in dogs only.

A necropsy with complete gross and microscopic examination was conducted on major organ systems of high-dose and control mice and for all dose levels of rats and dogs. Sections (5 μm) of paraffin-embedded tissues were prepared and stained with hematoxylin and eosin for microscopic evaluation.

Statistical evaluation

Overall effects of dose on body weights, body weight gains, and clinical pathology data in rodents were evaluated by one-way

Table 1 Design of toxicity studies with DOL10 given as a single i.v. bolus (*MS* moribund sacrifice)

Species	No. and sex per group	Doses μg/kg (μg/m^2)	Histopathology[a] (day evaluated, # animals)	Clinical pathology[b] (day evaluated)	Body weight (day evaluated)
Mouse	10M	0, 200, 300, 450, 675 (0, 600, 900, 1350, 2025)	Day 4, 5M; day 15, 5M	4, 15	–1, 1, 4, 8, 11, 15
Rat	10M/10F	0, 25, 75, 100, 225, 337.5 (0, 150, 450, 600, 1350, 2025)	Day 4, 5M/5F; day 29, 5M/5F	–5, 4, 8, 15, 22, 29	–5, –1, 1, 4, 8, 15, 22, 29
Dog	1M/1F	22.5, 45 (450, 900)	Day 15, 1M/1F (450 μg/m^2) Day 7/8, 1M/1F (MS, 900 μg/m^2)	–1, 2, 4, 8, 11, 15	–4, –1, 1, 4, 8, 15
Dog	2M/2F	0, 10, 20 (0, 200, 400)	Day 8, 1M/1F; day 31, 1M/1F	–3, 2, 4, 8, 15, 22, 29, 31	–3, 1, 2, 4, 8, 15, 22, 29, 31

[a] Day of sacrifice and histopathology as scheduled in the original study design. Histopathology was also performed on animals found dead or sacrificed in a moribund condition, but day of evaluation is shown only for dogs, because there were significant findings in this species
[b] Clinical pathology determinations were performed on the days shown; unscheduled clinical pathology determinations performed prior to moribund sacrifice are not shown

analysis of variance followed by Dunnett's test comparing each treated group with the respective control group (null hypothesis rejection, $P < 0.05$). In dog studies, statistical evaluation was not performed due to the small numbers of animals; prestudy values for each individual served as the control values.

Results

Mortality and clinical signs

Mice

One mouse in the 2025 μg/m^2 group died on day 4 and a second on day 7; no other deaths occurred. Treatment-related adverse signs were observed at doses ≥1350 μg/m^2 body surface area and included hypoactivity, rough coat, hunched posture, dyspnea, and tremors.

Rats

All rats from the 1350 and 2025 μg/m^2 groups died or were moribund-sacrificed within the first 8 days after treatment. On day 4, five rats of each sex were sacrificed from the 600 μg/m^2 group; three of five remaining male rats and two of five remaining female rats were found dead on days 7 to 10. No mortality occurred at doses below 600 μg/m^2. Treatment-related adverse signs were observed at doses ≥150 μg/m^2 and included hypoactivity, rough coat, hunched posture, dyspnea and ataxia.

Dogs

The two dogs in the 900 μg/m^2 group were euthanized in a moribund condition on day 7 (male) or 8 (female), whereas both dogs that had received 450 μg/m^2 survived. One of four dogs that had received 400 μg/m^2 was euthanized in a moribund condition on day 7, at which time it had an elevated body temperature (>41 °C). Adverse signs were observed at doses ≥450 μg/m^2 and in the one dog that received 400 μg/m^2 and became moribund. The predominant signs were hypoactivity, anorexia, diarrhea, dehydration, hunched posture, drooling, and foul-smelling breath. In the 450 μg/m^2 group, these signs generally appeared by day 6 or 7 and persisted through days 12–13 before the dogs returned to normal.

Effects on body weight

Mice

Mice in the 1350 and 2025 μg/m^2 groups had lost an average of 9% and 21% of their body weight, respectively, by day 8. Thereafter, both groups gained weight until terminal sacrifice. Body weights were unaffected at doses below 1350 μg/m^2.

Rats

On day 4, male and female rats in the 1350 and 2025 μg/m^2 dose groups had an average body weight loss of 12% relative to the control group. Surviving females continued to lose weight through day 8. In the 600 μg/m^2 dose group, males had a maximum body weight loss of 8% on day 8. Growth resumed thereafter, and by day 15 the group mean was not statistically different from the control group. No effects on body weight were observed in female rats in the 600 μg/m^2 dose group or in either sex at doses below 600 μg/m^2.

Dogs

At the time of moribund sacrifice, the male and female dogs in the 900 μg/m^2 dose group had lost 14% and 9% of their body weights, respectively, since day 1. The two dogs in the 450 μg/m^2 group had lost 6.5% of their body weight by day 8. The weight loss is consistent with the anorexia and diarrhea observed in these animals. No biologically relevant effects on body weight were observed at doses below 450 μg/m^2.

Hematology

Mice

WBC counts on day 4 indicated a mild leukopenia (Fig. 1a), although the mean values were not statistically different from control values. Lymphocytes exhibited the same dose-response relationship, that is counts were decreased 20–33% in the 1350 μg/m^2 dose group, coincident with the degree of leukocyte reduction (data not shown). Because lymphocytes account for almost 75% of the WBCs, the change in WBCs was attributed primarily to the lymphocyte component. In contrast, more pronounced decreases in neutrophils (83–100%), reticulocytes (100%), and platelets (29–49%) relative to control levels occurred at doses ≥300 μg/m^2. These changes were dose-related, as shown in Fig. 1b for neutrophil counts. By day 15, total WBC, neutrophil, platelet and reticulocyte counts were markedly elevated in the 2025 μg/m^2 group compared to control group values, but these parameters had recovered to normal levels in the 600, 900, and 1350 μg/m^2 dose groups. RBC counts were not affected by DOL10 at any dose level.

Rats

Moderate to marked decreases in leukocytes (52–90%, Fig. 1a), lymphocytes (32–82%), neutrophils (69–100%, Fig 1b), reticulocytes (74–96%, Fig. 2) and platelets (10–98%) were observed by day 4 or 8 at doses ≥600 μg/m^2. At the 450 μg/m^2 dose level, male rats also exhibited significant decreases in leukocytes (54%), neutrophils

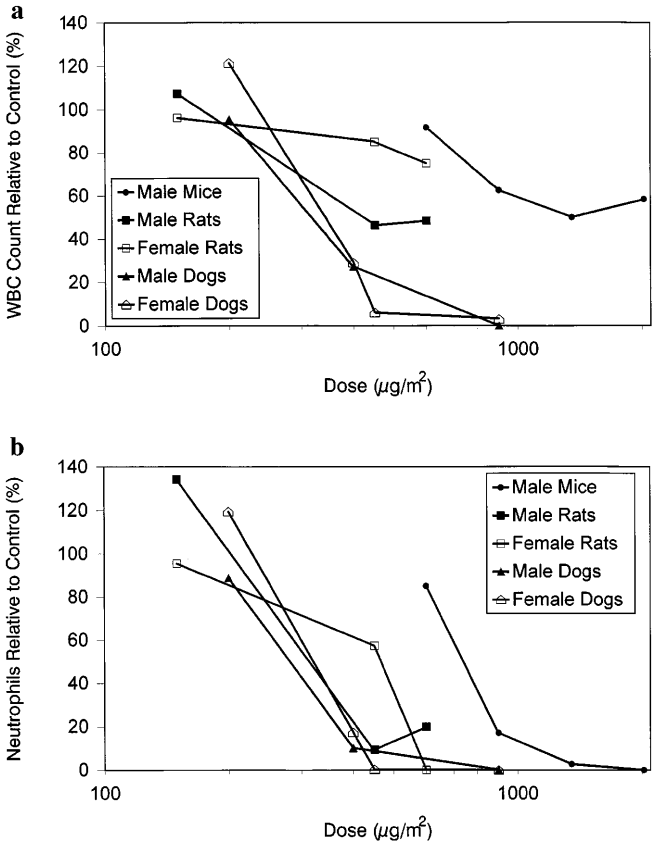

Fig. 1a, b Maximal effect of a single i.v. bolus dose of DOL10 on (a) WBC count and (b) neutrophil count in male CD2F1 mice (day 4), F-344 rats (day 8), and beagle dogs (day 8). For rodents, the data presented relate to control levels obtained on the same day as the WBC nadir; for dogs, the data presented relate to baseline values on day −3

(91%), and lymphocytes (48%) on day 8. The effect on females was less severe at the same dose level (Fig. 1b). In addition, a mild anemia (~20% decrease in RBC, HGB, and HCT) was observed in the 600 μg/m^2 dose group on day 8. By day 15, the leukopenia, neutropenia

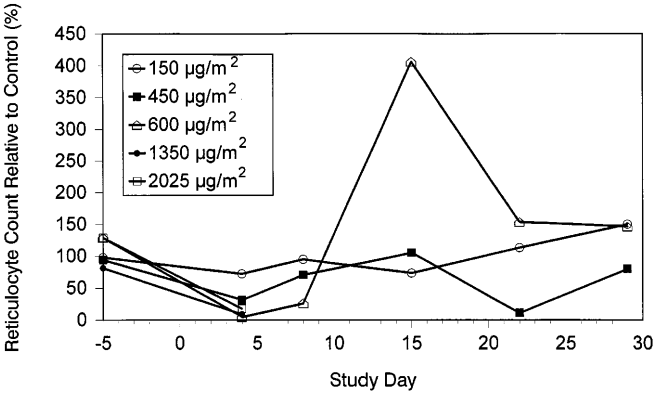

Fig. 2 Effect of a single i.v. bolus dose of DOL10 on reticulocyte count in male F-344 rats. The data are presented as percentage of control group mean values at each time point

and lymphocytopenia had resolved, but the regenerative anemia persisted (characterized by an elevation in MCV) and was accompanied by mild reticulocytosis (Fig. 2) and thrombocytosis in the 600 μg/m^2 group. No hematologic effects were observed in the 150 μg/m^2 dose group at any time or in the other dose groups on day 22.

Dogs

Dogs that received doses ≥400 μg/m^2 exhibited moderate to marked decreases in leukocytes (50–97%) due to severe neutropenia, lymphocytopenia, monocytopenia and eosinopenia. WBCs were almost completely absent in the peripheral blood (WBC count 0.4–1.0 × 10^3/μl) in the 450 and 900 μg/m^2 groups on day 8 (Fig. 1a). Thereafter, the WBC count and related parameters recovered to within normal levels in surviving animals by day 11. In addition, the female dog from the 900 μg/m^2 group and both dogs from the 450 μg/m^2 group developed normocytic, normochromic anemia as evidenced by moderate decreases in RBC, HGB, and HCT (18–32%) during the second week after dosing. The male dog from the 900 μg/m^2 group had moderate thrombocytopenia (platelets decreased by 46% relative to the predose value); this dog was euthanized in a moribund condition on day 7, possibly before anemia could develop. No hematologic abnormalities were evident in dogs that had received the 200 μg/m^2 dose.

Serum chemistry

Mice

No treatment-related effects on serum chemistry parameters were observed in mice.

Rats

Increases in BUN (4- to 6-fold), AST (16- to 35-fold), ALT (5- to 14-fold), and glucose (2- to 3-fold) were evident in both males and females that had received the 1350 or 2025 μg/m^2 dose; smaller increases in AST and ALT were apparent in male rats in the 600 μg/m^2 dose group. No biologically relevant serum chemistry effects were observed at nonlethal doses.

Dogs

ALP was elevated 3- to 11-fold and hypoglycemia was present in the dogs at lethal or near-lethal doses (450 and 900 μg/m^2). The hypoglycemia was attributed to dehydration and reduced food intake observed in these animals.

Histopathologic findings

Table 2 summarizes the microscopic lesions observed in the bone marrow of all three species.

Mice

Microscopic evaluation was performed on tissues from the 2025 μg/m^2 and control groups only. Mice necropsied on day 4 exhibited depletion of femoral bone marrow, which was characterized as a marked decrease in the numbers of erythroid and myeloid cells and a moderate decrease in the number of megakaryocytes. By day 15, there was a moderate increase in the numbers of myeloid cells, probably due to a compensatory hyper-

Table 2 Summary of histopathologic findings in bone marrow. No bone marrow lesions were observed after day 15; observations from later time-points are not included. Only histology findings are shown, but cytology evaluations confirmed these results. Depletion occurred in the erythroid, myeloid and megakaryocyte populations with identical severity and occurrence, except as indicated. Data presented are number of animals affected/total number of animals, with range of severity in parentheses (1 = minimal, 2 = mild, 3 = moderate, 4 = marked)

	DOL10 dose (μg/m^2)								
	0	150	200	400	450	600	900	1350	2025
Mice (day 4)									
Depletion, femur	0/5								4/4 (3–4)
Increased no. of myeloid cells, femur	0/5								0/5
Mice (day 15)									
Depletion, femur	0/5								0/5
Increased no. of myeloid cells, femur	0/5								4/4 (3)
Rats (day 4 and unscheduled deaths[b]; male and female combined)									
Depletion, femur	0/10	0/10			9/10[a] (3–4)	14/14 (4)		20/20 (4)	19/19 (2–4)
Dogs (day 8 or 15 and unscheduled deaths[b]; male and female combined)									
Depletion, sternum and rib	0/2		0/2	1/2 (4)	0/2		2/2 (4)		
Hyperplasia, sternum and rib	0/2		0/2	0/2	2/2 (2)		0/2		

[a] Myeloid and erythroid depletion occurred in 4/5 animals, and megakaryocyte depletion occurred in 3/5 animals; however, in all cases the severity was 3–4

[b] Histopathology evaluation was performed on animals sacrificed on the day indicated and also on animals sacrificed in a moribund condition or found dead

plasia in response to the earlier myelotoxicity. Mice in this group also exhibited a moderate diffuse atrophy of the thymus and an absence of cytoplasmic vacuolization of hepatocytes.

Rats

Doses ≥450 μg/m^2 were associated with markedly lower numbers of erythroid and myeloid cells and megakaryocytes in the bone marrow. The myelotoxicity was observed on day 4 but had resolved by day 29. There was also mild diffuse lymphoid depletion of the mesenteric lymph node on day 4 (450 and 600 μg/m^2 dose groups) and day 29 (600 μg/m^2 group only) and marked diffuse depletion of extramedullary hematopoietic cellular elements in the spleen on day 4 in the 450 μg/m^2 group.

One of five rats of each sex in the 600 μg/m^2 dose group had minimal lesions in the ileum on day 4. However, all the animals that were moribund or found dead (doses ≥600 μg/m^2) had more extensive and severe lesions in the entire gastrointestinal tract. Other lesions included histiocytosis in the mesenteric lymph node and spleen and renal protein casts; these were observed only at lethal dose levels.

Dogs

Microscopic lesions were found in several organs from all dogs in the 450 and 900 μg/m^2 groups and in the one moribund dog from the 400 μg/m^2 group, but not in the three remaining dogs from this dose group or dogs in the 200 μg/m^2 group. Effects on the bone marrow were prominent in dogs that were moribund, and were characterized by marked depletion of megakaryocytes and erythroid and myeloid cells at doses of 400 and 900 μg/m^2 (Table 2). Dogs in the 450 μg/m^2 group were sacrificed 2 weeks after treatment, and by that time mild hyperplasia of the bone marrow was observed, indicating bone marrow recovery with mild overcompensation following drug-induced toxicity. Recovery was also indicated in the 400 μg/m^2 group by the absence of bone marrow lesions in surviving animals on day 31.

Diffuse lymphoid cell depletion (moderate to marked) was observed in the spleen at doses ≥400 μg/m^2, and in the bronchial, mandibular, and mesenteric lymph nodes (mild to marked) and the palatine tonsils (marked) at doses of 400 and 900 μg/m^2. Lymphoid depletion was not observed in the lymph nodes and tonsils of dogs from the 450 μg/m^2 group, probably because these animals were sacrificed later and the tissues had recovered. The DOL10-induced lesions in the palatine tonsils were quite impressive. There was marked necrosis and cellular depletion of lymphoid follicles with concurrent severe diffuse necrotic inflammation that extended deep into the neighboring connective tissues surrounding the tonsils. In addition, the tonsils had abundant secondary bacterial growth, visible as multifocal yellow foci on the surface at necropsy.

Other lesions observed in moribund-sacrificed dogs were marked thymic atrophy and lower amounts of extramedullary hematopoiesis present in the liver and spleen relative to control dogs. Hepatocytic vacuolization, which is typically present in the liver, was not present in these dogs.

Table 3 Summary of major findings (*MTD* highest dose that produced severe toxicity without lethality, *ND* not determined, *NOEL* no observable effect level)

Species	MTD[a] μg/m^2 (μg/kg)	NOEL μg/m^2 (μg/kg)	Primary target organs[a]
Mouse	1350 (450)	900 (300)	Bone marrow, thymus
Rat	450 (75)	ND	Bone marrow, spleen
Dog	≤400 (≤20)	200 (10)	Bone marrow, lymphoid organs, tonsils

[a] Only those organs that were affected at doses below or equal to the estimated MTD are listed. Organs that were affected only at lethal doses are not included

Discussion

Table 3 summarizes the essential observations from these studies. The MTD of DOL10 was determined to be 1350 μg/m^2 in mice, 450 μg/m^2 in rats, and ≤400 μg/m^2 in dogs. Rats and dogs exhibited similar sensitivity to DOL10, whereas mice were approximately three- to six-fold less sensitive. At the MTD levels, the primary target organ in all three species was the bone marrow; species-dependent cell depletion in the thymus, spleen, lymphoid organs, and/or tonsils were also noted microscopically, coincident with effects on the bone marrow cells.

The most severe and clinically relevant effect produced by DOL10 was myelotoxicity, which was dose-limiting in all three species. This effect was expected for DOL10 based on in vitro studies which have shown it to be a potent inhibitor of hematopoietic progenitor cell proliferation [10]. The myelotoxicity was characterized by a pancytopenia, with the neutrophils being the most severely affected (Fig. 1b). The decreases in WBC numbers originated from the marked cellular depletion in the bone marrow (Table 2). The effects on myeloid cells were transient since both WBC count and bone marrow cellularity had recovered approximately 2 weeks after treatment. A compensatory hyperplasia was observed in mice and dogs in response to the demand for more blood cells following depletion of the cellular elements of the marrow. In addition to the myelotoxicity, reticulocytes were markedly lower or absent in all three species, and rats and dogs showed evidence of mild anemia, with this condition resolving within 1–2 weeks. Thus, DOL10 produced a generalized effect on bone marrow myeloid/erythroid precursor cells with the effect on myeloid cells having greater consequence biologically.

Myelotoxicity is a commonly observed feature of antineoplastic agents that bind tubulin [6, 7, 19]. However, DOL10 offers a clinical advantage over other tubulin-binding drugs because the schedule of administration is a single i.v. bolus and the vehicle is phosphate buffer, pH 7. Other tubulin-binding drugs require administration as an infusion, i.e. vinblastine [21] or the vehicle is troublesome, i.e. those that contain cremophore EL or Tween 80 [4, 9, 11].

Recent studies have shown a good correlation between results from in vitro bone marrow toxicity assays of granulocyte/macrophage colony formation (CFU-GM) and in vivo toxicity when myelotoxicity in dose-limiting [15, 16]. When differences in sensitivity to bone marrow toxicity exist between rodents and dogs, data from CFU-GM assays from these species and from human donors is useful to gain an insight into which animal model more closely predicts bone marrow toxicity for humans [20]. The IC_{90} values for myelotoxicity in CFU-GM assays are 10 p*M* for human, 4.5 p*M* for canine and 8000 p*M* for murine progenitor cells following a 1-h exposure to DOL10 (Schweikart KM et al., manuscript in preparation). Based on these in vitro results, murine hematopoietic progenitor cells are 1800-fold less sensitive than canine cells. In contrast, mice are three- to six-fold less sensitive to DOL10-induced myelotoxicity than dogs. The difference in sensitivity to DOL10 between murine and canine myeloid progenitor cells is at least 300-fold greater than the corresponding difference in sensitivity between these species in vivo; the reason for this difference in sensitivity is unknown but may be due to rapid clearance of the drug in vivo.

Preliminary pharmacology studies using radiolabeled DOL10 have shown that the drug has a very short half-life in mice following i.v. administration, and by 15 min only 10% of the total plasma radioactivity is associated with the parent drug [14, 18]. Unfortunately, plasma levels of unlabeled DOL10 were below the limit of quantitation for the available HPLC assay, and therefore pharmacokinetic parameters for rats and dogs could not be determined in the present studies. However, protein binding is similar in mouse, dog, and human plasma [14] and so the amount of free drug available to produce a biological effect is comparable between the three species.

Besides the bone marrow, DOL10 was toxic to the lymphoid organs, thymus, and tonsils. Necrosis and cellular depletion of lymphoid follicles in the tonsils of dogs is likely the reason some of the dogs had difficulty swallowing. Abundant bacterial growth flourished in the affected tonsils and was probably responsible for the dogs' foul-smelling breath. Moderate to marked atrophy of the thymus was also present in mice and dogs. Although thymic involution or atrophy is normal in aging rodents and dogs, the degree of atrophy observed in the groups treated with DOL10 was more severe than would be expected for untreated animals of this age, and a similar increase in thymic involution was not evident in any of the vehicle-treated animals. At the MTD or higher doses, lymphoid cell depletion in the lymph nodes and/or spleen occurred in rats and dogs and extramedullary hematopoiesis (EMH) was observed in the spleen and/or liver of all three species. EMH is usually composed of predominantly myeloid elements with large numbers of megakaryocytes and numerous erythroid elements [8]; it is believed to be a compensatory response to the bone marrow toxicity. Other effects of DOL10 included gastrointestinal toxicity, hepatocellular vacuolization, and renal protein casts. These lesions were seen only at lethal doses and appear to have little, if any, clinical relevance.

DOL10 recently completed phase I clinical trials and the dose-limiting toxicity is granulocytopenia [13], in agreement with these preclinical studies in animals. Six of 15 patients also had mild peripheral sensory neuropathy, which appeared to be more frequent in patients with underlying peripheral neuropathies prior to the initiation of the clinical trial. In our study, some mice and dogs exhibited ataxia. However, this occurred only at doses that were lethal and therefore ataxia (which can be a crude indicator of neurotoxicity) was not considered to be clinically relevant.

In summary, DOL10 produced dose-limiting myelotoxicity in mice, rats and dogs. Mice were approximately three- to six-fold less sensitive to the toxic effects of DOL10 than dogs and rats. The IC_{90} for human CFU-GM is reported to be 10 p*M*, which is comparable to the IC_{90} value of 4.5 p*M* for canine CFU-GM, suggesting that the toxicity results from dogs would be predictive of the effects in humans. The lowest dose at which toxicity was observed in dogs was 200 $\mu g/m^2$. Based on these results, the recommended clinical starting dose for DOL10 is one-third this dose, or 65 $\mu g/m^2$. This dose was found to be nontoxic in clinical trials.

These preclinical toxicology studies correctly predicted that myelotoxicity would be dose-limiting, and according to McElroy et al. [13], the area under the curve strongly correlated with the degree of leukopenia that was observed in the phase I clinical trial. Moreover, the human MTD appears to be in the range 325–455 $\mu g/m^2$, which is comparable to the MTD in dogs (almost 400 $\mu g/m^2$). Thus, the in vitro and in vivo results correctly predicted that the sensitivity of humans would be comparable to that of dogs and not mice.

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