Cancer Chemother Pharmacol (1996) 38: 129–135

ORIGINAL ARTICLE

G. Sylvester Price · Rodney L. Page
J. Edmond Riviere · J. Mark Cline · D.E. Thrall

Pharmacokinetics and toxicity of oral and intravenous lonidamine in dogs

Received: 8 October 1993/Accepted: 9 October 1995

Abstract Plasma lonidamine concentration and toxicity were investigated in dogs receiving 100, 200, 400, 800, 1200 mg/m^2 orally twice daily for 30 days and in dogs receiving single intravenous doses of 200, 400, 800, 1200 mg/m^2. Physical or laboratory signs of toxicity were not observed in dogs receiving oral lonidamine, but severe vomiting and signs of acute hepatic and pancreatic toxicity were observed in dogs receiving intravenous doses that exceeded 400 mg/m^2. The area under the lonidamine concentration versus time curve (AUC) in dogs receiving 200, 400, and 800 mg/m^2 of lonidamine intravenously was a 1.8-, 3.3-, and 8.7-fold higher than in dogs receiving oral lonidamine. This suggests that the bioavailability of oral lonidamine may be limited. However, centrilobular hepatocellular swelling and vacuolation were observed in dogs receiving oral lonidamine. Serum alanine aminotransferase (ALT) activity was increased in dogs receiving intra-venous lonidamine. These findings suggest that lonidamine is hepatotoxic in dogs. However, serum ALT was increased in only 1/4 dogs receiving 400 mg/m^2 of lonidamine intravenously and plasma concentration were within the range capable of sensitizing hyperthermia and chemotherapy. Therefore, this dose and route appears to be a viable and controllable method for prospective quantification of lonidamine interaction with systemic chemotherapy and/or hyperthermia.

Supported in part by Grant Nos. CA29582 and CA 08344 (NRSA) awarded by DHHS/NIH

G.S. Price (✉) · R.L. Page
Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606, USA
Fax (919)-829-4336

J.E. Riviere · J.M Cline · D.E. Thrall
Department of Anatomy, Physiologic Sciences and Radiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606, USA

Key words lonidamine · pharmacokinetics · dog

Introduction

Lonidamine is an indazole carboxylic acid derivative which enhances the cytotoxicity of radiation [18, 21–23, 27], and chemotherapy [5, 10, 25, 26, 30, 35, 39]. Furthermore, lonidamine has been recently shown to reverse multidrug resistance [4]. The mechanisms of these effects are incompletely understood, but potentiation may be attributable to bioenergetic and membrane alterations induced by lonidamine. Lonidamine was originally investigated as an antispermatogenic agent [8, 32] and was shown to inhibit mitochondrial energy metabolism [14] by altering membrane structure [12]. Presumably, lonidamine inhibits repair of potentially lethal damage induced by radiation [18, 23, 27] and doxorubicin efflux [15] because both are energy-requiring processes. Doxorubicin uptake is also enhanced by lonidamine [15]. This effect may be the result of lonidamine-induced alterations in plasma membrane structure.

Lonidamine is only slightly cytotoxic at 37°C but has enhanced cytotoxicity at elevated temperature [6, 17, 20, 33]. The mechanism of this effect has not been thoroughly investigated, but hyperthermia may increase susceptibility to lonidamine-induced energy inhibition by altering mitochondrial structure [33]. Regardless of the mechanism of interaction, results of recent studies suggest that hyperthermia and lonidamine may be combined to enhance the effects of radiation [27] or chemotherapy [5].

Humans receiving lonidamine, which is typically given orally, experience myalgia, nausea, vomiting, somnolence, lethargy and cutaneous hyperesthesia [1, 9, 24, 29, 36, 37]. Although these reactions are not life-threatening, they are dose-limiting [1, 9, 24, 29, 36, 37]. Furthermore, peak plasma lonidamine concentrations in many patients [9, 36, 37] are variable and are also

often below concentrations which have been shown to be necessary to potentiate chemotherapy [5, 10, 25, 26, 30, 35] and hyperthermia [17, 20]. Lonidamine sensitization and hence response rates may increase if higher or more reliable plasma lonidamine concentrations could be achieved. Additionally, very little is known regarding lonidamine pharmacokinetics in humans or other species. Therefore, a better understanding of the relationships between lonidamine dose, plasma concentrations, and toxicity may suggest administration methods that would decrease toxicity and/or produce higher plasma concentrations in humans.

The initial purpose of this study was to characterize lonidamine toxicity, identify the maximum tolerable dose of lonidamine and correlate these findings with plasma lonidamine concentrations in dogs receiving oral lonidamine. However, our initial observations suggested that it was difficult to induce toxicity in dogs. Therefore, the study was modified to include an investigation of lonidamine toxicity and plasma concentrations in dogs receiving lonidamine intravenously. Results from this study will be helpful in designing prospective lonidamine trials in dogs and other species, and may facilitate a more complete understanding of available data in humans.

Materials and methods

Oral lonidamine

Treatment groups

Normal adult mongrel dogs (11.3–27.6 kg) received 100, 200, 400, 800 or 1200 mg/m^2 of lonidamine every 12 h for 30 days. There were three dogs in each group. Lonidamine tablets (150 mg) were supplied by Angelini Pharmaceuticals, River Edge, N.J.

Plasma lonidamine concentrations

Dogs were fasted for 12 h before the first day of treatment. Heparinized blood was collected every 15 min for the first hour, and 2, 4, and 6 h after treatment. Samples were stored at -70°C until analyzed. Lonidamine concentrations were measured by high-pressure liquid chromatography (HPLC). Plasma was mixed 1:1 with 30% acetonitrile, 30% ethanol, and 5% triethylamine and filtered through a 30 000 Da cutoff filter unit (Amicon, Danvers, Mass). The filtrate was injected (Waters WISP 710B, Milford, Mass) onto a reverse phase octadecylsilane (C-18) column (22 cm × 4.6 mm, 5 μm spherical particles; Brownlee Laboratories, Santa Clara, Calif.) and eluted isocratically (1 ml/min) with 0.05 *M* ammonium acetate in 50% acetonitrile and 0.6% acetic acid at 50°C. Effluent absorbance was measured at 300 nm (Waters LC spectrophotometer, Milford, Mass.). Lonidamine eluted at approximately 6 min and concentrations were quantified (Waters 730 Data Module and 721 system controller, Milford, Mass.) by comparing the chromatogram area of the filtrates to the area of 5 μg lonidamine/ml diluted in 30% acetonitrile, 30% ethanol, and 5% triethylamine. Recovery was 77.8% (coefficient of variation = 9.2%), the limit of detection was 0.05 μg/ml and, lonidamine chromatogram area versus concentration (0.1–100 μg/ml) was linear ($r^2 = 0.9998$).

Maximal concentration (C_{max}) and time of maximal concentration (T_{max}) were determined by visual inspection of plasma concentration-time data. The area under the concentration versus time curve (AUC) was calculated from zero time to infinity (AUC_0^{∞}) using the trapezoidal method [16].

Toxicity

Pretreatment and weekly toxicity evaluations consisted of physical examination, body weight measurement, ECG, differential blood count, prothrombin time, activated partial thromboplastin time, and fibrinogen concentration, serum biochemical profile (alanine aminotransferase (ALT), alkaline phosphatase, amylase, bilirubin, BUN, creatinine, glucose, electrolytes), and urinalysis. Normal reference range values for canine hematology, serum biochemistry and urinalysis were used to identify biochemical and hematologic toxicity. Signs of lonidamine-induced myalgia, cutaneous hyperesthesia and testicular discomfort were tested by observing responses to aggressive muscle, skin and testicle palpation. Dogs were also monitored for signs of lameness, gait abnormalities, and gastrointestinal toxicity (anorexia, vomiting or diarrhea).

Dogs were sacrificed on day 30 by an overdose of intravenous pentobarbital sodium. Postmortem examinations were performed and the following tissues were collected: brain, myocardium, lung, thyroid, liver, pancreas, stomach, small intestine, colon, kidney, adrenal and thyroid glands, ovary/testicle, spleen, lymph node, bone marrow, skeletal muscle, and skin. Tissues were preserved in phosphate-buffered 10% formalin, embedded in paraffin, sectioned (6 μm), and stained with hematoxylin and eosin. Since toxicity had not been observed by day 30, tissues from dogs treated with the highest and lowest lonidamine dose were examined for histopathology. If histologic lesions were observed in these two dose groups, tissues from dogs treated with intermediate doses were also examined.

Intravenous lonidamine

Treatment groups

Lonidamine powder (Angelini Pharmaceuticals) was dissolved (10 mg/ml) in 0.2 *M* Tris(hydroxymethyl)aminomethane (Trizma base, Sigma Chemical Co., St. Louis Mo.) and 1.5 *M* glycine, and filtered (0.45 μm), immediately before injection. Normal adult mongrel dogs (8.8–27.4 kg) were fasted for 12 h before receiving bolus injections of 200, 400, 800 or 1200 mg/m^2 of lonidamine. One additional group received vehicle only. The volume of vehicle administered was equivalent to that administered to the 1200 mg/m^2 dose group. There were four dogs in each group.

Plasma lonidamine concentrations

Lonidamine concentrations were measured in heparinized blood collected every 20 min for the first hour and, 2, 4 and 6 h after treatment. The plasma lonidamine concentration at zero time (C_0) was extrapolated from nonlinear regression of concentration-time data. Plasma lonidamine AUC was calculated as described above.

Toxicity

A differential blood count, serum biochemical profile (as described above), serum creatine phosphokinase, and urinalysis were obtained before treatment. In dogs receiving 200, 400, and 800 mg/m^2 of lonidamine, these tests were repeated 6 h after treatment (concurrent

with the final plasma lonidamine sampling). However, dogs receiving 1200 mg/m^2 suffered intractable emesis, so these dogs, and dogs receiving vehicle only, were examined for toxicity and sacrificed by pentobarbital overdose, 1 h after lonidamine was administered.

Dogs receiving 200, 400, and 800 mg/m^2 of lonidamine were sacrificed by pentobarbital overdose 6 h after lonidamine administration. Since, the intravenous lonidamine study was intended to generate plasma concentration data, postmortem examinations were not planned. However, they were performed on dogs that had received 800 and 1200 mg/m^2 of lonidamine because severe gastrointestinal and biochemical toxicities were observed. Tissues were processed as described above.

In vitro hemolysis

The plasma of dogs receiving intravenous lonidamine was observed to be hemolyzed. Therefore, erythrocytes from two additional normal dogs were exposed in vitro to vehicle or lonidamine (0.5, 1.0, 2.0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 or 1000 μg/ml). Hemolysis was assessed visually.

Statistical analysis

Data are reported as the mean ± standard error of the mean (SEM). Analysis of variance [34] was used to test the effect of dose and route of administration on lonidamine C_{max}, T_{max} and, AUC. A P-value less than <0.05 was considered significant.

Results

Oral lonidamine

Plasma concentrations

Concentration versus time profiles in dogs receiving oral lonidamine are shown in Fig. 1. Terminal lonidamine decay was erratic in 4/15 dogs. In 1/3 dogs receiving 100 mg/m^2 of lonidamine, plasma concentration was unchanged for two consecutive periods during the last 4 h of sampling. In 3/3 dogs receiving 800 mg/m^2, the terminal lonidamine concentration was 1.5-fold higher than the penultimate concentration. Lonidamine C_{max}, T_{max}, and AUC increased as dose increased (Table 1).

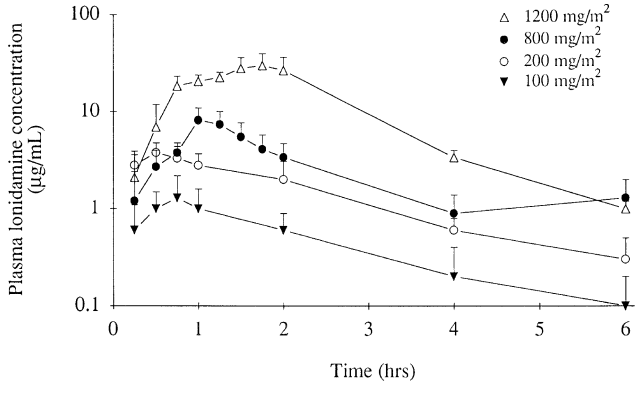

Fig. 1. Mean plasma lonidamine concentration profiles in dogs ($n = 3$/dose) receiving single oral doses of lonidamine. The 400 mg/m^2 dose group was omitted for clarity

Toxicity

Physical or laboratory signs of toxicity were not observed during the 30-day treatment period. However, there were histologic lesions in the liver and testes. Centrilobular hepatocellular swelling and vacuolation were observed in all dogs (Fig. 2A). Spermatogenic arrest and tubular atrophy were observed in the testes (Fig. 2B). Spermatids were released prematurely and were karyomegalic and multinucleate. Spermatogonia were nonmitotic and there were decreased numbers of primary and secondary spermatocytes, spermatids and spermatozoa.

Intravenous lonidamine

Plasma concentrations

Concentration versus time profiles in dogs receiving intravenous lonidamine are shown in Fig. 3. Terminal lonidamine decay was erratic in 2/12 dogs. In 1/4 dogs receiving 200 mg/m^2 of lonidamine, the plasma concentration was unchanged for two consecutive periods during the last 4 h of sampling. In another dog receiving 200 mg/m^2 of lonidamine, lonidamine was not detected in the penultimate sample whereas the terminal concentration was 0.1 μg/ml. Dogs receiving 1200 mg/m^2 of lonidamine were sacrificed 1 h after treatment because of intractable emesis. Therefore plasma concentration and AUC data are available for dogs receiving 200, 400, and 800 mg/m^2 of lonidamine (Table 2). The AUC in dogs receiving lonidamine intravenously was 1.8- to 8.7-fold higher than in dogs receiving lonidamine orally (Fig. 4). Moreover, this route-dependent difference in AUC increased as a function of dose ($P < 0.04$).

Table 1 Kinetic parameters in normal dogs ($n = 3$/dose) receiving oral lonidamine. (C_{max} peak plasma lonidamine concentration, T_{max} time of maximal plasma lonidamine concentration, AUC area under the concentration versus time curve). All parameter values increased significantly ($P < 0.01$) in a dose-dependent manner. Values are means ± standard error

Dose (mg/m^2)	Dose (mg/kg)	C_{max} (μg/ml)	T_{max} (min)	AUC (μg h/ml)
100	4	1.6 ± 0.8	30 ± 9	4.3 ± 1.5
200	8	3.9 ± 1.1	35 ± 5	9.3 ± 3.8
400	15	5.9 ± 1.2	60 ± 30	15.3 ± 4.0
800	30	8.7 ± 2.3	50 ± 10	20.5 ± 7.1
1200	50	34.5 ± 7.1	100 ± 13	66.2 ± 15.0

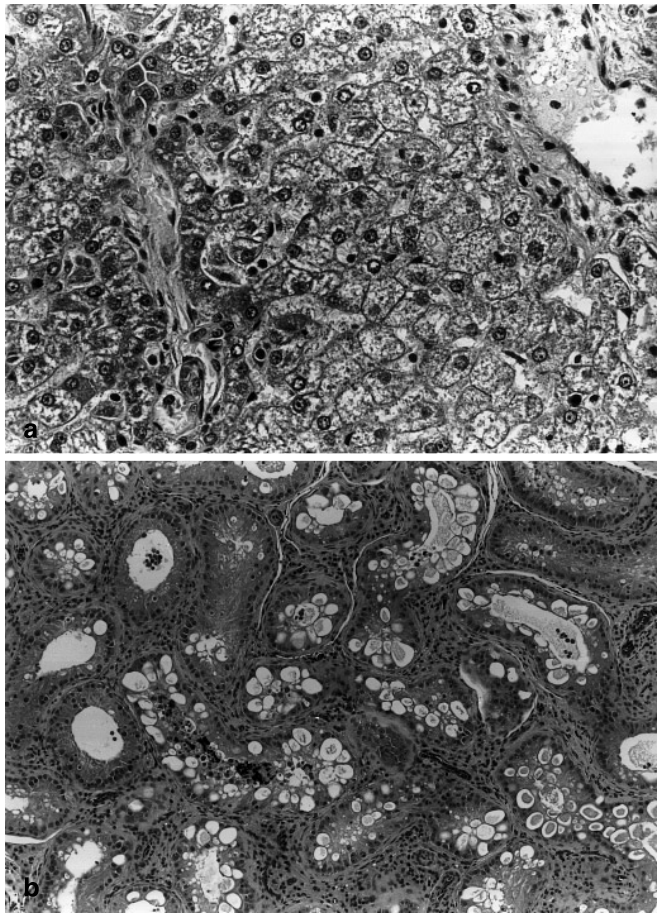

Fig. 2. Representative histologic lesions observed in dogs receiving oral lonidamine on the 30th day of treatment (X400). **A** Centrilobular hepatocellular swelling and vacuolation in a dog receiving 200 mg/m^2 twice daily for 30 days. **B** Spermatogenic arrest and tubular atrophy in a dog receiving 800 mg/m^2 twice daily for 30 days

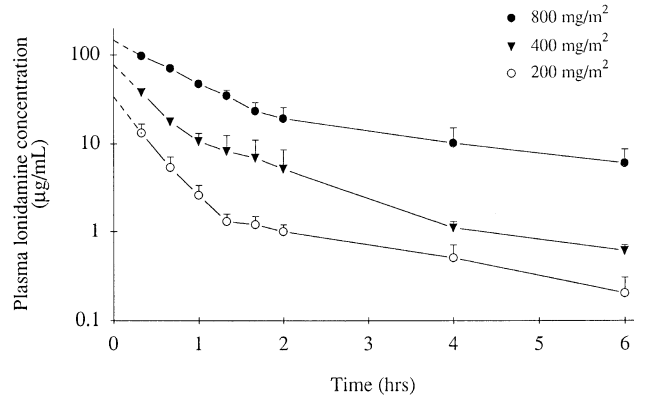

Fig. 3. Mean plasma lonidamine concentration profiles in dogs ($n = 4$/dose) receiving single intravenous doses of lonidamine

Toxicity

Lonidamine induced vomiting, hemolysis and increased serum ALT and amylase activity (Table 3).

Table 2 AUC in normal dogs ($n = 4$/dose) receiving intravenous lonidamine (AUC area under the concentration versus time curve, C_0 plasma concentration at zero time). Values are means ± standard error

Dose (mg/m^2)	Dose (mg/kg)	C_0 (μg/ml)	AUC (μg h/ml)
200	8	34.1 ± 7.7	17.0 ± 3.9
400	15	77.8 ± 11.0	50.0 ± 7.5
800	30	148.2 ± 10.9	179.1 ± 27.4

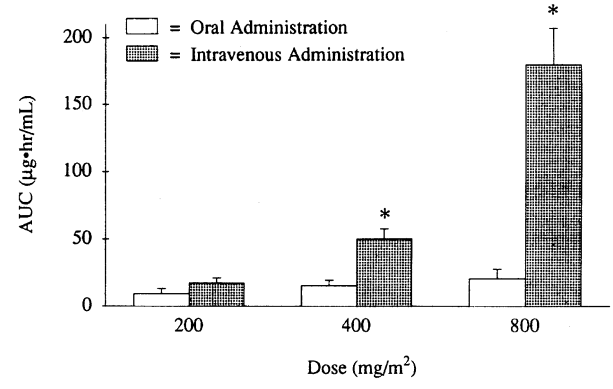

Fig. 4. Area under the concentration versus time curve (AUC) in dogs receiving either oral or intravenous lonidamine. *$P < 0.02$ vs oral administration

Eight of 16 dogs receiving lonidamine vomited within 1 h of treatment. Multiple episodes were observed in 1/4, 2/4 and 4/4 dogs receiving 400, 800 and, 1200 mg/m^2 of lonidamine, respectively.

Plasma samples obtained for pharmacokinetic analysis were hemolyzed but post-treatment hematocrit was unchanged. Subjectively, hemolysis in dogs receiving 200, 400, or 800 mg/m^2 of lonidamine was mild relative to dogs receiving 1200 mg/m^2. Hemolysis was maximal in initial samples (20 min after dosing) and resolved within 6 h. In vitro exposure of erythrocytes from untreated dogs to lonidamine concentrations greater than 125 μg/ml instantly induced visible hemolysis. Although the vehicle did not induce hemolysis in vitro, mild hemolysis was noted 1 h after the vehicle had been administered in 1/4 dogs.

Serum ALT increased approximately sixfold in 1/4 and 2/4 dogs receiving 400 and 800 mg/m^2 of lonidamine, respectively. In 1/4 dogs receiving 800 mg/m^2 of lonidamine, serum amylase activity and bilirubin concentration increased 2.5- and 5-fold, respectively.

Hepatic and testicular tissues of dogs receiving 800 or 1200 mg/m^2 were histologically normal. However, ecchymotic hemorrhages were observed in the pancreas of 3/4 and 2/4 dogs receiving 800 and 1200 mg/m^2 of lonidamine, respectively. Acute peripancreatic fat necrosis, presumably due to pancreatic enzyme leakage, was observed in 1/4 dogs receiving 1200 mg/m^2 of lonidamine.

Table 3 Characteristics and frequency of toxicity observed 6 h after single intravenous dose of lonidamine in normal dogs ($n = 4$/dose) (*ALT* serum alanine aminotransferase activity, *H* = marked hemolysis interfered with assay)

Dose (mg/m^2)	Vomiting	Hemolysis	Elevated ALT (>45 IU/l)	Elevated bilirubin (≥0.6 mg/dl)	Elevated amylase (>1480 IU/l)
200	0/4	2/4	0/4	0/4	0/4
400	1/4	1/4	1/4	0/4	0/4
800	3/4	0/4	2/4	1/4	1/4
1200[a]	4/4	4/4	H	H	0/4
Vehicle[a]	0/4	1/4	0/4	0/4	0/4

[a] Toxicity assessed 1 h after treatment

Discussion

Vomiting and signs of acute hepatic and pancreatic toxicity were observed in dogs receiving intravenous lonidamine whereas acute toxicity was not observed in dogs receiving oral lonidamine. This apparent route-dependent toxicity may be attributable to the fact that lonidamine AUC was 1.8- to 8.7-fold higher when the drug was administered intravenously. Because two different groups of dogs were used sequentially to study differences between oral and intravenous lonidamine, lonidamine bioavailability could not be calculated. However, the marked difference in AUC between dogs receiving lonidamine by oral and intravenous routes suggests that oral bioavailability was poor. Hepatic extraction may have limited lonidamine plasma concentrations in dogs receiving oral lonidamine. However, differences in AUC between dogs receiving oral and intravenous lonidamine increased as a function of dose (Fig. 4), suggesting that absorptive mechanisms may be saturable. Results of studies in other species support this hypothesis. In rats [31], mice [33], monkeys [31] and humans [3, 36, 37], peak lonidamine concentrations plateau as oral lonidamine dose increases. In vitro data show that lonidamine uptake becomes nonlinear at concentrations exceeding 16 μg/ml [15]. Therefore, it is possible that this phenomenon causes gastrointestinal absorption of lonidamine to become saturated. Alternatively, the physicochemical properties of lonidamine may cause its solubility within the gastrointestinal tract to decrease with increasing dose.

Fluctuations in terminal plasma lonidamine concentration decay were observed regardless of whether lonidamine was administered orally or intravenously. Similar fluctuations have been observed in humans receiving lonidamine [3]. These fluctuations suggest that lonidamine may undergo enterohepatic recirculation [13]. In the present study, plasma samples were collected during the first 6 h after treatment. Therefore, the AUC reported here is underestimated. Ideally, frequent plasma samples should have been collected during the latter half of the treatment interval. Nevertheless, enterohepatic recirculation occurred regardless of administration route, so it is unlikely that this phenomenon influenced the observed route-dependent difference in AUC.

The C_{max} in dogs following oral administration of lonidamine is three- to six-fold lower than in humans receiving a comparable dose [36, 37]. Therefore, the absorptive capacity of dogs appears to be less than that in humans. Alternatively, species differences in disposition may also be responsible for this disparity. However, clearance and volume of distribution data are not available for humans and the short sampling period used in the present study preclude estimation of kinetic parameters. Elimination half-life data may help determine whether the administration interval used in the present study was insufficient to induce steady-state drug concentrations. However, the apparent dose-dependent bioavailability of oral lonidamine may limit practical and reliable investigation of efficacy and toxicity in dogs.

Although clinical signs of toxicity were not observed in dogs receiving oral lonidamine, there were histologic lesions in the liver and testes. Lonidamine-induced testicular lesions have been described [11, 19] but hepatic lesions have not been reported. The mechanism of this hepatopathy is unknown although lonidamine-induced changes in plasma or mitochondrial membrane structure may be responsible for the observed morphologic changes. Reversible hepatic enzyme elevations have been observed in humans receiving lonidamine [1, 36]. However, histologic evaluation of these hepatic effects has not been reported.

The ALT and bilirubin abnormalities observed in dogs receiving intravenous lonidamine are compatible with acute hepatocellular damage [7]. Histologic abnormalities were not observed in these dogs. However, hepatic tissue samples were collected within 6 h after drug exposure so histologic lesions may not have developed. The mechanism of this hepatotoxicity is unknown. It is reasonable to speculate that the acute hepatotoxicity is related to the cause of the hepatopathy in dogs receiving oral lonidamine. Liver enzyme elevations were not observed in dogs receiving oral lonidamine presumably because their lonidamine concentrations were substantially lower than in dogs receiving intravenous lonidamine. Studies investigating whether lonidamine-induced hepatopathy is irreversible and/or progressive are warranted.

The immediate hemolytic effect we observed in vitro suggests that lonidamine induces intravascular hemolysis. Lonidamine causes dose-dependent alterations in erythrocyte shape and plasma membrane structure in vitro [12]. Therefore, the hemolysis observed in the present study is probably attributable to lonidamine-induced changes in membrane structure. Hemolysis was not observed in dogs receiving oral lonidamine, but plasma concentrations in these dogs were lower than in dogs receiving intravenous lonidamine. Mild hemolysis was observed in 1/4 dogs receiving vehicle. The vehicle used to dissolve lonidamine may have contributed to hemolysis induced by lonidamine, but the vehicle did not induce hemolysis in vitro. Therefore, the cause of hemolysis observed in 1/4 dogs receiving vehicle only is unknown. Regardless of the cause, hemolysis resolved within 6 h after lonidamine administration, and therefore is unlikely to be clinically significant. However, the long-term effects of this toxicity should be investigated.

The vomiting observed in dogs receiving intravenous lonidamine may have resulted from stimulation of the chemoreceptor trigger zone. Alternatively, lonidamine may have induced acute pancreatitis. Serum amylase was elevated in 1/4 dogs receiving 800 mg/m^2, ecchymotic hemorrhages were observed in the pancreas of 5/8 dogs receiving 800 and 1200 mg/m^2, and peripancreatic fat necrosis was observed in 1/4 dogs receiving 1200 mg/m^2.

Even though the results reported here were not generated using a crossover design, our findings suggest that poor bioavailability may limit the use of oral administration to investigate the efficacy and toxicity of lonidamine in dogs. Moreover, plasma concentrations in dogs receiving 100–800 mg/m^2 lonidamine orally were below those capable of sensitizing hyperthermia [17, 20, 28, 38] and chemotherapy [2, 5, 39] in vitro. Higher plasma lonidamine concentrations may be achieved by more frequent administration. However, this may be impractical when lonidamine is used to sensitize hyperthermia and chemotherapy patients because treatments are administered intermittently. Plasma concentrations in dogs receiving 400 mg/m^2 of lonidamine were within the range capable of sensitizing hyperthermia [17, 20, 28, 38] and chemotherapy [2, 5, 39] in vitro. Therefore, it may be more suitable to administer lonidamine intravenously when attempting to sensitize hyperthermia and chemotherapy. Furthermore, intravenous administration may circumvent variable and suboptimal concentrations currently observed in humans [9, 36, 37]. Additional studies are warranted to determine whether 400 mg/m^2 is the maximal tolerable dose in dogs. Doses higher than 400 mg/m^2 were associated with hepatic and pancreatic toxicity and severe emesis in dogs. Therefore, 400 mg/m^2 is a reasonable starting intravenous dose to use in prospective canine clinical studies.

References

1. Band PR, Maroun J, Pritchard K, Stewart D, Coppin CM, Wilson K, Wisenhauer EA (1986) Phase II study of lonidamine in patients with metastatic breast cancer: A National Cancer Institute of Canada Clinical Trials Group study. Cancer Treat Rep 70:1305
2. Bellelli A, Bellelli L, Di Palma M, Lorenzon I, Mattioni M, Nista A, Pavese I, Rusconi V, Sezzi ML (1990) Effects of VM-26 and lonidamine on a B16 melanoma cell line. Anticancer Res 10:565
3. Besner J-G, Leclaire R, Band PR, Deschamps M, De Sanctis AJ, Catanese B (1984) Pharmacokinetics of lonidamine after oral administration in cancer patients. Oncology 41 [Suppl 1]:48
4. Calabresi R (1994) Drug resistance: lonidamine. In: Rosenberg SA (ed) Principles and practice of oncology, vol 8. JB Lippincott Co, Philadelphia, p 1
5. Chitnis M, Adwankar M (1986) Potentiation of adriamycin cytotoxicity in P388 murine leukemia sensitive and resistant to adriamycin by use of lonidamine and hyperthermia. Tumori 72:469
6. Chitnis M, Juvekar A, Adwankar M, Advani S (1986) Inhibition of DNA systhesis by lonidamine and hyperthermia in human chronic myeloid leukemia cells. Anticancer Res 6:749
7. Coles EH (1986) Veterinary clinical pathology, In: Liver function. 4th edn. WB Saunders, Philadelphia, p 129
8. Coulston F, Dougherty WJ, LeFevre R, Abraham R, Silvestrini B (1975) Reversible inhibition of spermatogenesis in rats and monkeys with a new class of indazol-carboxylic acids. Exp Mol Pathol 23:357
9. De Angelis LM, Currie VE, Kim J-H, Krol G, O'Hehir MA, Farag FM, Young CW, Posner JB (1989) The combined use of radiation therapy and lonidamine in the treatment of brain metastases. J Neurooncol 7:241
10. De Fabritiis P, Sandrelli A, Covelli A, Simone F, De Felice L, Pulsoni A, Zupi G, Mandelli F (1989) In vitro pharmacological purging of human bone marrow is enhanced by lonidamine. Exp Mol Pathol 50:210
11. De Martino C, Malorni W, Bellocci M, Floridi A, Marcante ML (1981) Effects of AF 1312TS and lonidamine on mammalian testis. A morphologic study. Chemotherapy 27 [Suppl 1]:27
12. De Martino C, Malorni W, Accinni L, Rosati R, Nista A, Formisano G, Silvestrini B, Arcancia G (1987) Cell membrane changes induced by lonidamine in human erythrocytes and T-lymphocytes, and Ehrlich ascites tumor cells. Exp Mol Pathol 46:15
13. Dobrinska MR (1989) Enterohepatic circulation of drugs. J Clin Pharmacol 29:577
14. Floridi A, De Martino C, Marcante ML, Apolloni C, Scorza Barcellona P, Silvestrini B (1981) Morphological and biochemical modifications of rat germ cell mitochondria induced by new antispermatogenic compounds: studies in vivo and in vitro. Exp Mol Pathol 35:314
15. Floridi A, Gambacurta A, Bagnato A, Bianchi C, Paggi MG, Silvestrini B, Caputo A (1988) Modulation of adriamycin uptake by lonidamine in Ehrlich ascites tumor cells. Exp Mol Pathol 49:421
16. Gibaldi M (1991) Biopharmaceutics and clinical pharmacokinetics. In: Compartmental and noncompartmental pharmacokinetics, 4th edn. Lea & Febiger, Philadelphia, p 14
17. Greco C, Cavaliere R, Giovanella B, Natali PG, Zupi G (1987) Effect of sequential application of hyperthermia and chemotherapy on the survival of a thermoresistant human melanoma cell line. Cancer Biochem Biophys 9:223
18. Hahn GM, Kersen I van, Silvestrini B (1984) Inhibition of the recovery from potentially lethal damage by lonidamine. Br J Cancer 50:657

19. Heywood R, James RW, Barcellona PS, Campana A, Cioli V (1981) Toxicological studies on 1-substituted-indazole-3-carboxylic acids. Chemotherapy 27 [Suppl 2]: 91
20. Kim JH, Kim SH, Alfieri A, Young CW, Silvestrini B (1984) Lonidamine: a hyperthermic sensitizer of HeLa cells in culture and of the Meth-A tumor in vivo. Oncology 41 [Suppl 1]: 30
21. Kim JH, Alfieri A, Kim SH, Young CW, Silvestrini B (1984) Radiosensitization of Meth-A fibrosarcoma in mice by lonidamine. Oncology 41 [Suppl 1]: 36
22. Kim JH, Alfieri AA, Kim SH, Young CW (1986) Potentiation of radiation effects on two murine tumors by lonidamine. Cancer Res 46: 1120
23. Kim JH, Kim SH, He SQ, Alfieri AA, Young CW (1989) Potentiation of radiation effects on multicellular tumor spheroids (MTS) of HeLa cells by lonidamine. Int J Radiat Oncol Biol Phys 16: 1277
24. Kokron O, Maca S, Scheiner W, De Gregorio M, Ciottoli GB (1984) Phase II study of lonidamine in inoperable non-small-cell lung cancer. Oncology 41 [Suppl 1]: 86–89
25. Ning S, Hahn G (1990) Cytotoxicity of lonidamine alone and in combination with other drugs against murine RIF-1 and human HT1080 cells in vitro. Cancer Res 50: 7867
26. Raaphorst GP, Feeley MM, Heller DP, Danjoux CE, Martin L, Maroun JA, De Sanctis AJ (1990) Lonidamine can enhance the cytotoxic effect of cisplatin in human tumour cells and rodent cells. Anticancer Res 10: 923
27. Raaphorst GP, Feeley MM, Danjoux CE, Martin L, Maroun J, DeSanctis AJ (1991) The effect of lonidamine (LND) on radiation and thermal responses of human and rodent cell lines. Int J Radiat Oncol Biol Phys 20: 509
28. Raaphorst GP, Feeley MM, Martin L, Danjoux CE, Maroun J, Desanctis AJ, Ko D (1991) Enhancement of sensitivity to hyperthermia by Lonidamine in human cancer cells. Int J Hyperthermia 7: 763
29. Robins HI, Longo WL, Lagoni RK, Neville AJ, Hugander A, Schmitt CL, Riggs C (1988) Phase I trial of lonidamine with whole body hyperthermia in advanced cancer. Cancer Res 48: 6587
30. Rosbe KW, Brann TW, Holden SA, Teicher BA, Frei E III (1989) Effect of lonidamine on the cytotoxicity of four alkylating agents in vitro. Cancer Chemother Pharmacol 25: 32
31. Serge G, Catanese B (1981) Pharmacokinetics of lonidamine. Chemotherapy 27 [Suppl 1]: 77
32. Silvestrini B, Caputo A (eds) (1981) Lonidamine: a new pharmacological approach to the study and control of spermatogenesis and tumors. Chemotherapy 27 [Suppl 2]: 1
33. Silvestrini B, Hahn GM, Cioli V, De Martino C (1983) Effects of lonidamine alone or combined with hyperthermia in some experimental cell and tumor systems. Br J Cancer 47: 221
34. Steel RGD, Torrie JH (1980) Principles and procedures of statistics: a biometrical approach. In: Analysis of variance I: the one-way classification. 2nd edn. McGraw-Hill, New York, p 137
35. Teicher BA, Herman TS, Holden SA, Epelbaum R, Liu S, Frei E (1991) Lonidamine as a modulator of alkylating agent activity in vitro and in vivo. Cancer Res 51: 780
36. Weinerman BH, Eisenhauer EA, Besner J-G, Coppin CM, Stewart D, Band PR (1986) Phase II study of lonidamine in patients with metastatic renal cell carcinoma: A National Cancer Institute of Canada Clinical Trials Group study. Cancer Treat Rep 70: 751
37. Young CW, Currie VE, Kim JH, O'Hehir MA, Farag FM, Kinahan JE (1984) Phase I and clinical pharmacologic evaluation of lonidamine in patients with advanced cancer. Oncology 41 [Suppl 1]: 60
38. Zupi G, Natali PG, Balduzzi MA, Greco C, di Filippo F, Silvestrini B, Caputo A (1983) Cytotoxic effect of lonidamine combined with hyperthermia on human melanoma cells in vitro. Relevance of time-sequence schedules of treatment. In: Spitzy KH, Karrer K (eds) Proceedings of the 13th International Congress of Chemotherapy, H. Egerman, Vienna, part 290, p. 1
39. Zupi G, Greco C, Laudonio N, Benassi M, Silvestrini B (1986) In vitro and in vivo potentiation by lonidamine of the antitumor effect of adriamycin. Anticancer Res 6: 1245