

Prospective evaluation of the safety of compounded bulk material L-asparaginase in dogs with lymphoma

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Use of compounded L-asparaginase became routine in veterinary oncology when manufacturing of Elspar[®] was discontinued in 2012. The objective of this study was to evaluate the safety of compounded L-asparaginase (CLASP, KRS Global Biotechnology, Boca Raton, FL, USA) in comparison with Elspar[®] (Lundbeck LLC, Deerfield, IL, USA). In addition, we documented the response to CLASP in combination with a corticosteroid in this population of dogs with lymphoma. Dogs were prospectively treated with 10 000 IU/m² CLASP or Elspar[®] subcutaneously. Corticosteroids were administered concurrently. Adverse events (AE) were assessed according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events v1.1 (VCOG-CTCAE). Response was recorded. Seventy-three dogs received 75 treatments (CLASP, *n* = 47; Elspar[®], *n* = 28). No AE were attributed to CLASP. Grade I and II AE probably or possibly related to treatment were observed following two Elspar[®] treatments. The overall response rate to the combination of CLASP and a corticosteroid was 80% (24% CR and 56% PR). In combination with a steroid, the compounded L-asparaginase evaluated in this study is safe and demonstrates activity against canine lymphoma. In the face of the discontinuation of Elspar[®], veterinarians should seek compounded LASP products that have been tested for activity, purity, and sterility.

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INTRODUCTION

The enzyme L-asparaginase (L-asparagine hydrolase, LASP) is important in the treatment of dogs with lymphoid neoplasia. It is effective, results in a rapid response, and is associated with a low incidence of adverse events (AE) (Rogers, 1989). L-asparaginase depletes circulating L-asparagine, a nonessential amino acid, by hydrolyzing it to aspartic acid and ammonia. Neoplastic lymphoid cells lack asparagine synthetase and are therefore dependent on extracellular sources. Depletion of asparagine by LASP results in decreased protein synthesis and cell death. This unique mechanism of action spares normal cells, resulting in a favorable safety profile (Valerius *et al.*, 1999; Chabner & Friedmann, 2011).

In December 2012, Lundbeck LLC, the sole manufacturer of Food and Drug Administration (FDA)-approved native L-asparaginase (trademarked as Elspar[®]), discontinued production of this drug. Other FDA-approved LASP products (e.g., Erwinaze[®]; Jazz Pharmaceuticals plc, Philadelphia, PA, USA and Oncaspar[®]; Sigma-Tau Pharmaceuticals, Inc., Gaithersburg, MD, USA) remain available in the United States, but are cost prohibitive for routine veterinary use. As a result, veterinary oncologists have turned to compounding pharmacies and FDA-registered outsourcing facilities for LASP. Compounded LASP preparations differ from each other based on the initial bulk material used, as well as variations in compounding practices. Consequent alterations in activity, purity, sterility, and the amount of bacterial endotoxin present have the potential to

result in adverse events (AE) and/or decreased efficacy (Ho *et al.*, 1970).

As clinicians anticipating use of compounded LASP in our patients, it was our goal to determine whether this was a safe replacement for Elspar®. The objective of this study was to evaluate adverse events associated with compounded LASP (CLASP, KRS Global Biotechnology, Boca Raton, FL, USA) in dogs with lymphoma. In addition, we documented the response to CLASP in combination with a corticosteroid in this population of dogs.

METHODS

This was a nonrandomized, open label, prospective cohort study that occurred at the University of Georgia, College of Veterinary Medicine (UGA) and BluePearl Georgia Veterinary Specialists (GVS). Cases were enrolled at both sites from initiation of the study until the supply of Elspar® (Lundbeck LLC, Deerfield, IL, USA) at UGA was depleted. Inclusion criteria were (i) histologic or cytologic diagnosis of large cell lymphoma and (ii) owner willingness to return for re-evaluation 3–7 days post-treatment. Although the treatment protocol and LASP used were the standard at each institution, owners were informed about the study and owner consent to recheck as scheduled and fill out a quality of life form was obtained verbally. Dogs with lymphoma that was naïve to treatment, relapsed, or refractory were eligible for inclusion. Dogs previously treated with LASP were included unless they had an allergic reaction to LASP or their lymphoma was resistant to LASP. Because of the potential difficulty distinguishing AE from clinical signs of lymphoma, dogs with confirmed or suspected cutaneous or gastrointestinal lymphoma were excluded.

Treatment

At GVS, dogs were treated with CLASP (KRS Global Biotechnology, Boca Raton, FL, USA), and at UGA, dogs were treated with Elspar®, based on the product available at each treatment site. L-asparaginase was administered at a standardized dosage of 10 000 IU/m² subcutaneously up to a maximum of 10 000 IU. Dogs were monitored for signs of an allergic reaction for a minimum of 30 min after drug administration.

All dogs previously treated with LASP (CLASP or Elspar®) were treated with diphenhydramine 2 mg/kg intramuscularly 20 min prior to LASP administration. Dogs were discharged from the hospital with prednisone for administration at home at a standardized dosage of 1.5 mg/kg/day *per os*. Hospitalized dogs received dexamethasone sodium phosphate for injection intravenously at a dosage equivalent to the standardized prednisone dosage within the 24 h prior to or at the same time as LASP. These dogs, and dogs with resistant lymphoma that received prednisone at home within the 24 h before treatment with LASP (because steroid therapy was ongoing), were categorized as pretreated with steroids. Dogs that did not receive prednisone until after discharge from the hospital were categorized as not pretreated with steroids.

Patient assessment

Patient data were collected using a case reporting form and included age, weight, sex, and breed. The anatomic location, stage, substage, and immunophenotype were recorded if known. Complete clinical staging was not required for inclusion in this study. Prior treatments, including corticosteroids, were recorded. If LASP had been used previously, the source, route of administration, and dosage were recorded.

Assessment of adverse events and response

At the initial visit, dogs were assessed with a physical examination including the measurement of target lesions. Additional diagnostics were performed at the discretion of the attending veterinarian. Three to 7 days later, dogs were re-evaluated for the assessment of AE and treatment response. A quality of life form was completed by owners (Figure S1).

Adverse events were graded, and relationship to LASP (vs. lymphoma, pre-existing conditions, concomitant medications, or other causes) was attributed as definitive, probable, possible, unlikely, or unrelated by the attending veterinarian according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events v1.1 (VCOG-CTCAE) (VCOG, 2011). Constitutional and gastrointestinal signs were attributed as definitively, probably, or possibly related to LASP if onset followed treatment and as unrelated or unlikely related if they were pre-existing at the time of treatment and continued post-treatment (i.e., attributed to lymphoma or other pre-existing conditions). Adverse events were managed at the discretion of the attending veterinarian. Response to treatment was assessed using the Veterinary Cooperative Oncology Group Response Evaluation Criteria for Peripheral Nodal Lymphoma in Dogs (v.1.0) (Vail *et al.*, 2010). For patients with extranodal lymphoma, response was assessed using WHO criteria: CR, complete disappearance of all disease; PR, ≥50% reduction but not 100% reduction in disease; PD, ≥25% increase in disease; SD, did not meet criteria for CR, PR, or PD (Owen, 1981). Overall response rate (ORR) was defined as the percentage of dogs achieving CR or PR.

Statistical analysis

Normality was determined using the D'Agostino-Pearson method. Treatment groups (CLASP vs. Elspar®) were compared using a t-test for weight and chi-square or Fisher's exact tests for categorical variables. Significance was set at $\alpha \leq 0.05$.

RESULTS

A total of 75 LASP treatments (CLASP, $n = 47$; Elspar®, $n = 28$) were administered to 73 dogs (Fig. 1). Case enrollment occurred from November 2013 to January 2015. Patient characteristics, prior treatments, and study treatment information are presented in Table 1. Results of clinical staging and

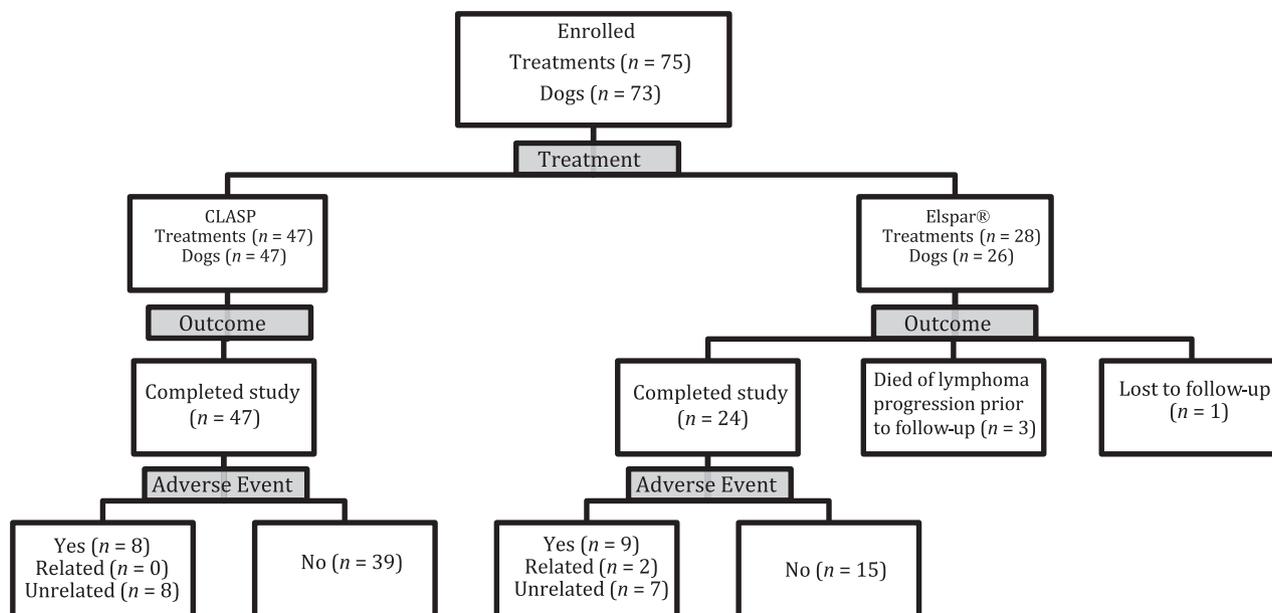


Fig. 1. Flow diagram of cases enrolled, the numbers of treatments administered, and outcome after treatment of 73 dogs with compounded L-asparaginase (CLASP, KRS Global Biotechnology, Boca Raton, FL, USA) or Elspar® (Lundbeck LLC, Deerfield, IL, USA) for lymphoma. A total of 75 treatments were administered to 73 dogs.

Table 1. Characteristics and prior treatment of 73 dogs with lymphoma treated with compounded L-asparaginase (CLASP, KRS Global Biotechnology, Boca Raton, FL, USA) or Elspar® (Lundbeck LLC, Deerfield, IL, USA)

	CLASP <i>n</i> = 47	Elspar® <i>n</i> = 26	<i>P</i> value*
Treatment site			
GVS	47		
UGA		26	
Median body weight (kg, with range)	28.7 (2–51.1)	26.6 (6.7–48.6)	0.92
WHO substage			
a	34	16	0.49
b	13	10	
Indication			
Naïve	42	18	0.07
Relapsed	0	2	
Refractory	5	6	
Pretreated with steroids			
Yes	8	15	0.0009
No	39	11	

*Significance set at *P* < 0.05.
 Bold value indicates significant value.

phenotypic analysis are not presented, as the information was not known in the majority of cases. A greater percentage of CLASP-treated dogs had lymphoma that was naïve to treatment (vs. relapsed or refractory to treatment; 89% vs. 69%); however, this did not reach statistical significance (*P* = 0.07). Four dogs (two treated with CLASP and two treated with Elspar®) had received LASP once previously. One dog treated with Elspar® in this study had received LASP twice previously.

All dogs were discharged with prednisone for administration at home. A corticosteroid was administered prior to or concurrently with LASP in 23 cases: 15 (CLASP, *n* = 7; Elspar®, *n* = 8) with relapsed or refractory lymphoma that were already on prednisone at the time of LASP treatment and 8 (CLASP, *n* = 1; Elspar®, *n* = 7) that were hospitalized for supportive care and therefore treated with injectable dexamethasone sodium phosphate. Significantly more Elspar®-treated dogs were already receiving prednisone or given concurrent injectable dexamethasone sodium phosphate (i.e., pretreated with steroid: Elspar®, 58% and CLASP, 17%, *P* = 0.0009).

Treatment and outcome are summarized in Fig. 1. Re-evaluation after treatment occurred as planned 3–7 days following 58 of 75 treatments. Six re-checks occurred earlier than planned (all at 2 days post-treatment), and seven occurred later than planned (median 8 days post-treatment, range 8–12 days). Aside from prednisone, none of dogs evaluated later than planned received additional anticancer therapy prior to re-evaluation. Three Elspar®-treated dogs were euthanized prior to re-evaluation 1–5 days after treatment. Death in all three dogs was attributed to progression of lymphoma, and no AE associated with Elspar® were observed. One dog treated with Elspar® failed to return for re-evaluation and was lost to follow-up. No immediate reaction to LASP was observed in this dog; however, information about AE after discharge and response assessment were not available.

Adverse events were observed following 17 of 75 (23%) treatments (Fig. 1). Categorization and attribution of all AE are presented in Table 2. None of the dogs treated with CLASP experienced AE attributed as definitely, probably, or possibly related to treatment. Of the five dogs that had previously received LASP, 1 CLASP-treated dog experienced an AE. This

Table 2. Adverse events (AE) observed following 47 treatments with compounded L-asparaginase (CLASP, KRS Global Biotechnology, Boca Raton, FL, USA) and 28 treatments with Elspar® (Lundbeck LLC, Deerfield, IL, USA) in 73 dogs with lymphoma. Categorization and attribution of AE were according to VCOG-CTCAE v1.1 (Vail *et al.*, 2010)

Treatment AE Category	Attribution (number of events)				
	Unrelated	Unlikely	Possible	Probable	Definite
CLASP (<i>n</i> = 8)					
Constitutional	1				
Dermatologic	1				
Gastrointestinal	5				
Renal/ Genitourinary		1			
Elspar® (<i>n</i> = 9)					
Constitutional	1		1*		
Gastrointestinal	4	2		2†	
Hepatobiliary/ Pancreas				1†	

*Grade 1 lethargy. †One dog with grade 2 pancreatitis resulting in grade 2 anorexia and grade 1 vomiting.

was grade 1 pruritus that was attributed as unrelated to treatment because it was pre-existing at the time of treatment. Two dogs treated with Elspar® experienced AE probably or possibly related to treatment. These were mild (grades 1 and 2) and resolved without sequelae. Specifically, one dog treated with Elspar® was lethargic for 12 h after treatment. Another dog experienced grade 2 anorexia and grade 1 vomiting 24 h after treatment with Elspar® and was diagnosed with grade 2 pancreatitis based on canine pancreatic lipase immunoreactivity (cPLI) test (Spec cPL®, Idexx Laboratories, Westbrook, ME, USA (582 µg/L, normal range <200 µg/L)). The dog was treated supportively with oral famotidine, metoclopramide, sucralfate, and maropitant citrate and was clinically normal within 48 h. The incidence of AE attributable to LASP was low in this study population and was not significantly different between dogs receiving CLASP vs. Elspar® ($P = 0.14$).

Response was assessed by lymph node measurements in all except for two dogs in which response was assessed based on circulating lymphoblasts on CBC. Response was unable to be assessed in one dog treated with Elspar® for spinal lymphoma with stable clinical signs (no motor function or sensation) after treatment. The overall response rate was 80% (11 CR and 26 PR) for CLASP and 59% (4 CR and 12 PR) for Elspar®. The response rate for dogs that were naïve to treatment was 83% (11 CR and 23 PR) for CLASP and 71% (3 CR and 9 PR) for Elspar®. The response rate for relapsed and refractory cases was 60% (3 PR) for CLASP and 40% (1 CR and 3 PR) for Elspar®. Response in dogs previously treated with LASP was PR ($n = 2$, CLASP), SD ($n = 1$, Elspar®), and PD ($n = 2$, Elspar®).

DISCUSSION

Based on our results, the L-asparaginase compounded by KRS Global Biotechnology between November 2013 and December

2014 was safe in the treatment of dogs with lymphoma. No AE were attributed to CLASP. In combination with a corticosteroid, CLASP showed activity against lymphoma with an overall response rate of 80%. As single agents, the reported response rates for corticosteroids and LASP are 48% and 73–79%, respectively (Bell *et al.*, 1984; Teske *et al.*, 1990; MacEwen *et al.*, 1992; Ogilvie *et al.*, 1994).

The compounded LASP evaluated in this study was nonsterile and required filtration prior to administration. It was not tested in-house by the outsourcing facility or by a third party, but was supplied with a certificate of analysis of the bulk raw material from Attix Pharmaceuticals (Toronto, ON, Canada) with the potency, purity, and an expiration date. Concern for patient safety prompted us to perform this study.

The most common adverse effects of LASP in dogs are IgE-mediated hypersensitivity reactions (Rogers, 1989; Saba *et al.*, 2009; Chabner & Friedmann, 2011). Signs include acute vomiting, diarrhea, facial swelling, urticaria, pruritus, and rarely, collapse due to anaphylactic shock. Previous studies report hypersensitivity reactions in 4–10% of dogs treated with LASP (MacEwen *et al.*, 1992; Saba *et al.*, 2009). We observed no immediate hypersensitivity reactions. However, because the majority of dogs received only a single dose of LASP and were therefore not sensitized to LASP, it is possible that reactions might have been seen with additional doses. In a study reporting hypersensitivity reactions in 10% of dogs treated with LASP, all of the dogs that reacted had received previous doses of LASP [median 3 (range, 2–6) doses of LASP before developing an allergic reaction] (Saba *et al.*, 2009). In addition, use of corticosteroids, and in cases that had previously received LASP, diphenhydramine, may have contributed to the lack of hypersensitivity reactions in this study. Pretreatment with diphenhydramine abrogates allergic reactions by blocking the effects of IgE-mediated histamine release (Rogers, 1989; Gustafson & Page, 2013). In one study, three dogs pretreated with diphenhydramine developed delayed allergic reactions within 24 h of discharge from the hospital, suggesting signs were ameliorated initially, but became evident as the antihistamine was cleared (Saba *et al.*, 2009). No similar delayed reactions were observed in this study; however, because all dogs received corticosteroids, these reactions could have been prevented. Corticosteroids are a standard component of induction protocols for canine lymphoma, and pretreatment with diphenhydramine after the first dose of LASP is routine. Although use of these medications potentially limited our evaluation of AE, we believed their use in a standardized manner was reasonable in this study to mimic the typical clinical setting.

Other adverse effects of LASP are thought to result from contamination with bacterial endotoxin or inhibition of protein synthesis secondary to asparagine depletion. These AE have been documented in people, but only rarely reported in dogs, and include pancreatitis, abnormal liver function tests, vague gastrointestinal signs, and decreased synthesis of proteins such as albumin and clotting factors (Rogers *et al.*, 1992; Ogilvie *et al.*, 1994; Chabner & Friedmann, 2011; Lyles *et al.*, 2011; Schleis *et al.*, 2011). One dog in this study was diagnosed with

pancreatitis following Elspar[®] treatment. Although pancreatitis has many potential etiologies (Hansen & Carpenter, 1983; Mansfield, 2012), this AE was attributed as probably related to Elspar[®] due to the timing of onset.

Assessment of AE was limited by the numbers of cases and the low incidence of AE associated with LASP. A sample size of over 500 cases would have been necessary to detect even a 100% difference in AE associated with LASP between the two groups with 80% power and 95% confidence. Because our supply of Elspar[®] was limited to 47 doses, we were unable to achieve this large number of cases. However, no AE were attributed to CLASP. In addition, as mentioned above, use of corticosteroids and diphenhydramine (for doses subsequent to the first dose of LASP) might have decreased the incidence and severity of AE. Another problem was the difficulty distinguishing the etiology of AE in some cases. Clinical signs were often nonspecific and potentially caused by LASP, response or progression of lymphoma, or competing factors. In designing this study, we made the decision to attribute signs to lymphoma if onset was prior to treatment with LASP. If clinical signs started after LASP, they were attributed as at least possibly related to LASP. While logical, this decision might have resulted in incorrect attributions and consequent over- or underestimation of the incidence of AE. A final limitation of this study was reliance on owners to report AE. If the information provided by owners was incomplete or inaccurate, over- or underreporting of AE might have been a consequence.

At the time this study was in process, the quality of compounded drugs was called to the attention of veterinary oncologists following the presentation of data suggesting quality control issues with compounded CCNU (Burton *et al.*, 2014). Potency testing found compounded CCNU only 50–54% as potent as CeeNU[®] (Bristol-Myers Squibb Company, Princeton, NJ). Although the observed response rate reported here suggests that CLASP is effective, no activity testing was performed. To the authors' knowledge, the activity of any compounded LASP product has not been reported previously in the literature.

In response to concerns about compounding drugs for veterinary use, in May 2015, the Food and Drug Administration (FDA) published a draft of a proposed guidance document on compounding animal drugs from bulk drug substances (<http://www.fda.gov/downloads/AnimalVeterinary/G%20GuidanceComplianceEnforcement/GuidanceforIndustr%20y/UCM446862.pdf>). This document proposes FDA policy regarding compounding by state-licensed pharmacies, state-licensed veterinarians, and facilities registered with the FDA as outsourcing facilities under section 503B of the Federal Food, Drug, and Cosmetic Act and will increase regulation of compounding drugs for veterinary use. It is important for veterinarians to be aware of this guidance and of the significance of 503B registration. The compounded LASP evaluated in this study was prepared by a registered outsourcing facility. Registered outsourcing facilities are expected to adhere to current good manufacturing practices (cGMP). In addition, these facilities are subject to inspections by the FDA that are vital for quality control. While the compounded drugs they supply are not FDA-approved, this

level of regulation provides some assurance to prescribers about the quality of the drug provided.

Because there are differences in sourcing of bulk material and compounding practices at different pharmacies and over time, multiple compounded chemotherapy preparations are produced. It is not feasible to clinically evaluate every compounded preparation of every chemotherapy drug for veterinary use. An important conclusion of this study is that veterinarians should use FDA-approved drugs whenever possible, and when not, veterinarians should seek compounded medications that have been evaluated for sterility, potency or activity (depending on which is appropriate for that drug type), and purity from pharmacies that are regulated and have had recent inspections by the FDA.

In conclusion, the compounded L-asparaginase distributed by the FDA-registered outsourcing facility, KRS Global Biotechnology, between November 2013 and December 2014 was safe and showed efficacy for treatment of dogs with lymphoma when used with prednisone or dexamethasone sodium phosphate. In the face of the discontinuation of Elspar[®], veterinarians should seek compounded LASP products that have been tested for activity, purity, and sterility.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Quality of life form filled out by owners of dogs with lymphoma treated with compounded L-asparaginase (CLASP, KRS Global Biotechnology, Boca Raton, FL, USA) or Elspar[®] (Lundbeck LLC, Deerfield, IL, USA).

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