Effects of L-asparaginase on Plasma Amino Acid Profiles and Tumor Burden in Cats with Lymphoma

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ABSTRACT

Asparaginase (Elspar®, Merck & Co., Inc., Whitehouse Station, NJ) is an enzyme derived from E. coli that depletes lymphoma cells of asparagine, inhibiting protein synthesis and resulting in cell death. It is currently unknown if feline lymphoma cells require asparagine for survival, and what impact Elspar® has on plasma amino acid levels in cats. Thirteen cats with confirmed LSA of any anatomic site were given one dose of Elspar® for treatment of their lymphoma. Plasma collected at 0, 2 and 7 days after Elspar® therapy was assayed using HPLC for asparagine, aspartate, glutamine, and glutamate levels. Ammonia levels were also measured at these time points. Cats were restaged 7 days after treatment to assess tumor response. Eight cats had T cell LSA, four cats had B cell LSA, and one cat's immunophenotype is unknown. Two complete responses (CR) and two partial responses (PR) to Elspar® were seen. Four cats clinically improved but had stable disease. Five cats had progressive disease (PD), four of which required additional chemotherapy during the 7 day study period. No drug-related adverse events occurred. Although not statistically significant, ammonia levels increased and asparagine levels decreased from baseline at 2 and 7 days post-treatment, respectively. Aspartate levels were significantly increased ($p < 0.05$) over baseline at 2 and 7 days post-treatment. Treatment with Elspar® did not significantly reduce asparagine levels within 7 days of treatment as occurs in dogs. The overall response rate (CR + PR) of feline lymphoma to Elspar® in this study was low (30%).

OBJECTIVES

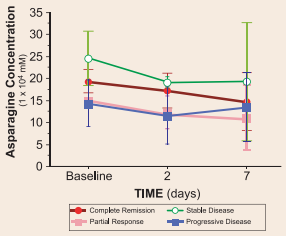
- ✔ Evaluate the response of cats with confirmed diagnosis of lymphoma to a single IM injection of L-asparaginase (Elspar®, Merck, Sharp & Dohme Inc., West Point, PA).
- ✔ Perform plasma amino acid profiles and ammonia levels prior to, 48 hours after, and 1 week after administration of the drug to assess changes in asparagine, aspartic acid, glutamine, and glutamic acid that occur with use of asparaginase in cats.

INTRODUCTION

Lymphoma is the most common cancer affecting cats. Cats with lymphoma do not enjoy the duration or quality of remission that dogs do, even with equivalent combination chemotherapy. L-asparaginase is a bacteria-derived enzyme that hydrolyzes asparagine to aspartic acid with the release of ammonia.[1] To a lesser extent glutamine is hydrolyzed to glutamic acid, as most asparaginases derived from E. coli possess 3-5% glutaminase activity.[2] Asparagine is a nonessential amino acid in mammals, as normal cells contain asparagine synthetase and can replace depleted stores.[2] L-asparaginase administration depletes asparagine, leading to decreased protein synthesis with subsequent cell death.[3] This mechanism of action makes L-asparaginase an ideal chemotherapeutic agent, as normal cells are generally unharmed. Moreover, L-asparaginase is minimally myelosuppressive and has few adverse effects.[4] Lymphoma cells are killed quickly, but any remaining cells can upregulate asparagine synthase for de novo asparagine production and become resistant to the effects of the drug after repeated doses.[2]

Although it is well accepted that L-asparaginase induces a fast, largely non-toxic tumor cell kill in canine lymphoma, its use in cats as a single agent is poorly documented. Asparagine levels quickly become non-detectable in treated dogs, with rebound after several weeks of L-asparaginase therapy.[1] The use of L-asparaginase as a rescue agent after combination chemotherapy in cats with mediastinal lymphoma resulted in a median survival of 8 weeks in one study.[5] L-asparaginase is currently part of the induction phase of some combination chemotherapy protocols, so the effect of this drug alone in treatment-naive lymphoma cannot be gleaned from previous studies.[6,7]

One of the unique characteristics of cats is a high demand for dietary protein compared to other species.[8] The serum concentrations of amino acids such as asparagine are highly dependent on the cat's diet and nutritional status.[8] Therefore, the overall higher-protein diets fed to cats may impact the ability of L-asparaginase to effectively deplete asparagine in the cat, thus leading to reduced tumor cell death when using this drug.



Graph 1: Plasma asparagine concentration (mean ± SD) and response before and after L-asparaginase administration to cats with lymphosarcoma.

METHODS AND MATERIALS

Thirteen cats with a confirmed diagnosis of lymphoma of any anatomic site with measurable disease and no prior therapy (including glucocorticoids) were enrolled in this study through the University of Tennessee College of Veterinary Medicine (UT-CVM) clinical oncology service. Routine tumor staging, including thoracic and abdominal imaging and flow cytometric or immunohistochemical immunophenotyping of the lymphoma, was performed. After a 12 hour fast, blood was drawn to measure baseline plasma ammonia, asparagine, aspartate, glutamine, and glutamate concentrations. A single dose of L-asparaginase was administered IM. Cats were reexamined in 2 days for blood sampling and measurement of amino acid levels. One week from drug administration, cats were reexamined for blood sampling and tumor restaging to assess response to therapy. The following criteria were used: Complete remission (CR)- 100% regression of measurable lesion(s); Partial remission (PR)- less than 100% but greater than 50% regression of disease, Stable disease (SD)- less than 25% regression and no new lesions, and no response (NR)- indicates no change or growth in size of the lesion(s). Ammonia concentrations were measured by the UT-CVM clinical pathology laboratory with normal cat plasma assayed simultaneously as control. Amino acid levels were measured with high-performance liquid chromatography (HPLC) by the UT-CVM clinical pharmacology laboratory. Statistical analysis with repeated measures ANOVA was used to assess changes in the cats' asparagine, aspartate, glutamine, and glutamate levels before and after L-asparaginase treatment. Patient-related factors such as anatomic site of disease, immunophenotype, and response to therapy were compared with amino acid levels with ANOVA to identify any association between these variables. Statistical significance was set at $p < 0.05$.

RESULTS

Eight cats had T cell LSA, four cats had B cell LSA, and one cat's immunophenotype is unknown. Two complete responses (CR) and two partial responses (PR) to Elspar® were seen. Four cats clinically improved but had stable disease. Five cats had progressive disease (PD), four of which required additional therapy (chemotherapy, steroids, radiation therapy) during the 7 day study period. No adverse reactions to Elspar® therapy were seen.

Ammonia levels were significantly increased from baseline at 2 and 7 days post-treatment ($p = 0.0393$). Asparagine levels were significantly decreased from baseline at 2 days ($p = 0.0319$) but not 7 days ($p = 0.0878$) post-treatment. Aspartate levels were significantly increased over baseline at 2 days ($p = 0.0011$) and 7 days ($p = 0.0015$) post-treatment. Glutamate levels were significantly increased at day 2 compared to day 7 post-treatment ($p = 0.0396$) but not compared to baseline ($p = 0.1788$) No significant changes in glutamine levels occurred in the 7 day study period. Low patient numbers in the response and anatomic site categories precluded detection of significant association between these variables and changes in amino acid concentrations.

Treatment with Elspar® significantly reduced asparagine levels within 2 days of treatment in cats, but this effect was lost at 1 week post-treatment. A significant correlation between reduction in asparagine levels and response to therapy could not be detected. The overall response rate (CR + PR) of feline lymphoma to Elspar® in this study was low (30%).

CONCLUSION

- ✔ Evidence exists that Elspar® causes a reduction in plasma asparagine levels in the cat at the current recommended dose, but this effect does not correlate with initial response to the drug and appears to be short-lived in this species. Possible causes for this phenomenon include the cat's innate ability to mobilize amino acids via urea cycle metabolism leading to prompt replenishment of asparagine stores, inherent asparagine synthase activity in feline lymphoma cells leading to poor overall response to therapy, or inadequate dosing of the drug to sufficiently deplete asparagine levels and induce tumor cell death. Adjustment of the currently recommended dose and/or dosing interval may be required to improve efficacy of this drug in cats with lymphoma.

ACKNOWLEDGEMENTS

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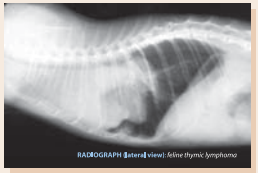
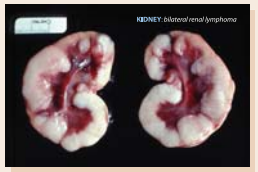
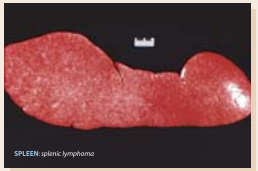
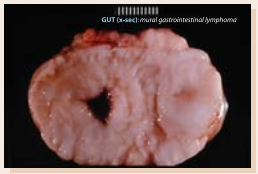
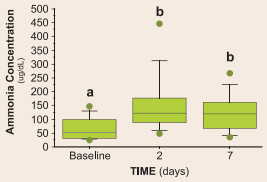


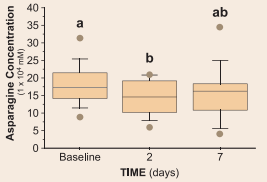




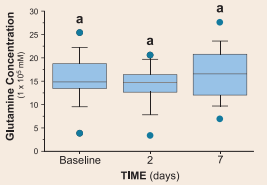




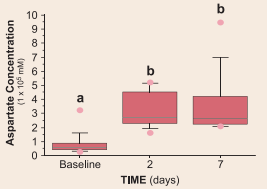
Graph 2: Plasma ammonia concentration before and after L-asparaginase administration to cats with lymphosarcoma. The superscripts denote difference at $p \leq 0.05$.



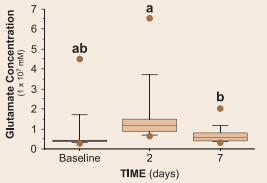
Graph 3: Plasma asparagine concentration before and after L-asparaginase administration to cats with lymphosarcoma. The superscripts denote difference at $p < 0.05$.



Graph 4: Plasma glutamine concentration before and after L-asparaginase administration to cats with lymphosarcoma. The superscripts denote difference at $p \leq 0.05$.



Graph 5: Plasma aspartate concentration before and after L-asparaginase administration to cats with lymphosarcoma. The superscripts denote difference at $p \leq 0.05$.



Graph 6: Plasma glutamate concentration before and after L-asparaginase administration to cats with lymphosarcoma. The superscripts denote difference at $p \leq 0.05$.

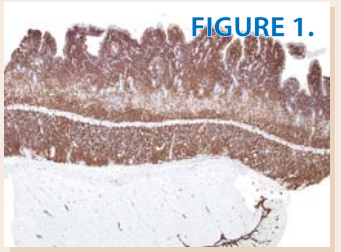


Figure 1: Section of jejunum from a cat (magnification 10X). The lamina propria, submucosal and intermuscular fascia is expanded by a neoplastic population of lymphocytes. Immunohistochemical staining of these lymphocytes for CD3 revealed strong diffuse expression, consistent with T-cell lymphosarcoma .

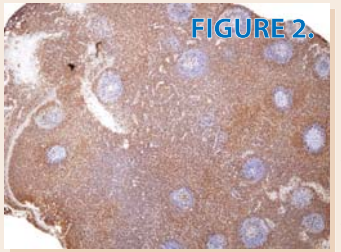


Figure 2: Section of mesenteric lymph node from the same cat as in *Figure 1* (magnification 4X). The paracortical regions of the lymph node are expanded and infiltrated by sheets of neoplastic lymphocytes. Immunohistochemical staining of these lymphocytes for CD3 revealed strong, diffuse, expression, consistent with T-cell lymphosarcoma. The B-cell germinal centers do not stain.

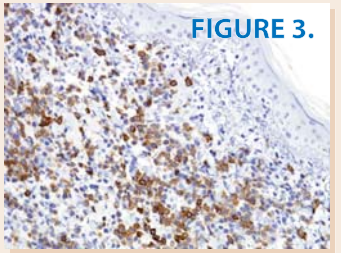


Figure 3: Section of stomach wall from a cat (magnification 10X). Normal tissue architecture of the stomach wall is obliterated by neoplastic lymphocytes. Immunohistochemical staining of these lymphocytes for CD79 revealed strong, diffuse, expression, consistent with transmural B-cell lymphosarcoma .

Figure 4: Section of a cutaneous mass from a cat (magnification 20X). The normal tissue architecture of the dermis and subcutis is obliterated by a neoplastic population of lymphocytes. These lymphocytes are seen focally within the surface epidermis. Immunohistochemical staining of these lymphocytes for CD3 revealed strong, diffuse, expression, consistent with T-cell lymphosarcoma.

REFERENCES

1- **Valerius KA, et al.** (1999) *JAVMA* 214: 353-356. Comparison of the effects of asparaginase administered subcutaneously versus intramuscularly for treatment of multicentric lymphoma in dogs receiving doxorubicin.

2- **Chabner BA.** Enzyme Therapy: L-asparaginase. In: Chabner BA and Longo DL, eds. **Cancer Chemotherapy and Biotherapy** Philadelphia, PA: Lippincott Williams & Wilkins; 2001: 647-656.

3- **MacEwen EG, et al.** (1992) *J Vet Intern Med* 6: 230-234. Evaluation of L-asparaginase polyethylene glycol conjugate versus native L-asparaginase combined with chemotherapy.

4- **Chun R, et al.** Cancer Chemotherapy. In: Withrow SJ and MacEwen EG, eds. **Small Animal Clinical Oncology.** Philadelphia, PA: W.B. Saunders Co.; 2001: 92-119.

5- **Jeglum KA, et al.** (1987) *JAVMA* 190: 174-178. Chemotherapy for lymphoma in 75 cats.

6- **Matus RE.** Chemotherapy of Lymphoma and Leukemia. In: Kirk RW, ed. **Current Veterinary Therapy X: Small Animal Practice.** Philadelphia, PA: Harcourt, Brace, Jovanovich, Inc.; 1989: 482-488.

7- **Vail DM, et al.** (1998) *J Vet Intern Med* 12: 349-354. Feline Lymphoma (145 Cases): Proliferation Indices, Cluster of Differentiation 3 Immunoreactivity, and their Association with Prognosis in 90 Cats.

8- **Kirk CA, et al.** Normal Cats. In: Hand MS, Thatcher CD, Remillard RL, Roudebush P, eds. **Small Animal Clinical Nutrition.** Topeka, KS: Mark Morris Institute; 2000: 291-347.