THE TREATMENT OF ELEVATED LIVER ENZYMES IN RETIRED RACING GREYHOUNDS USING AN ORALLY-ADMINISTERED NUTRACEUTICAL COMPOUND

A RETROSPECTIVE STUDY OF 64 CASES

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Abstract

Sixty-four retired racing greyhound dogs were found to have elevated alanine aminotransferase (ALT) upon admission to a non-profit canine blood donor facility (www.Hemopet.org)[1]. This group of dogs was administered a nutraceutical formulation (Hepato Support™)[2] to address the elevated ALT. These treated animals were retested for liver function by blood serum chemistry analysis after a minimum of 30 days on nutraceutical therapy. Results found a positive response rate in this treatment group with recorded 97% seroconversion to ALT values within normal limits defined by the reference laboratory (www.antechdiagnostics.com)[3]. The average decrease in ALT was 135.7 IU/L (median = 98 IU/L, decreases ranged from -69 IU/L to 850 IU/L and SD 150.4). Less dramatic decreases, but many of them still statistically significant, were seen in a number of other blood chemistry enzyme concentration changes. No adverse reactions were observed to this nutraceutical formulation in this study group during nutraceutical therapy.

Introduction

All blood donor dogs are greyhounds retired from the racing industry and kept in an isolated, closed colony environment at HemopetTM (www.hemopet.org), a private, non- profit company that supplies blood products to veterinarians throughout the United States, Canada, and Hong Kong.

Prior to arrival at Hemopet, available dogs are screened for blood type, organ function, including thyroid activity, and tick-borne, systemic fungal, and blood-transmissible diseases. When an individual's entry test for liver function is elevated, it is administered a nutraceutical medication for liver support2 and retested after a minimum of 30 days.

This retrospective study summarizes the hemograms and blood chemistry values of 64 dogs meeting the criteria for the study and selected by reviewing the results of nearly 700 total residents reviewed over a period of 7 years to evaluate the efficacy of Hepatosupport™ [2] in normalizing liver function test values.

Materials and Methods

Healthy adult Greyhound dogs (ages 1-5 years), recently retired from the racetrack, were adopted by a private, non-profit blood products company (www.hemopet.org) as blood donors, prior to their adoption as family companions. These dogs are pre-screened before acceptance into this closed colony for the "universal donor" blood type (DEA 4(C)), organ function, including thyroid activity, and tick-borne, systemic fungal, and blood-transmissible diseases. The current facility census as of January 2012 is 195 total residents. The facility is licensed and inspected annually under existing animal blood bank regulations of the State of California Department of Food and Agriculture. The facility holds Biologics License # 84 from this state agency, as it has since 1991.

All donor dogs are of blood type DEA 4(C) and are negative for all other known canine red blood cell antigens, including DEA 1.1 (A1), DEA 1.2 (A2), and DEA 7 (Tr), the antigens most associated with clinically significant transfusion incompatibilities in dogs. All donors receive on-site, 24 hour-a-day veterinary care, socialization, and maintenance. All donor dogs are current on immunizations for canine distemper, hepatitis, parainfluenza, leptospirosis, parvovirus, Bordetella, coronavirus and rabies virus.

All donor dogs have been blood and serologically tested for canine brucellosis, hemobartonellosis (hemoparasitic mycoplasma), Borrelia burgdorferi (Lyme disease), Dirofilaria immitis (heartworm disease), Ehrlichia canis, Rocky Mountain Spotted Fever, Leishmaniasis, Coccidioides immitis, Babesia canis, Babesia gibsoni, and plasma levels of von Willebrand factor.

The subset of Hemopet colony dogs identified as hypothyroid, based on established diagnostic criteria for sight hounds, is treated with twice daily thyroxine as prescribed by the veterinary professional staff. In cases where liver enzyme concentrations are elevated, the dogs are given nutraceutical support and treated with daily administration of Hepato Support™ [2].

This retrospective study examined blood tests kept on file at Hemopet for the residents of this blood donor colony over a period of 7 years (2004-2011).

Individuals were included in this study if their blood tests upon entry to the colony had liver function tests at least 10% above the upper range of normal limits defined by the reference laboratory that analyzed the blood samples (Antech Diagnostics™)[3]. Study participants were administered Hepato Support™ , a proprietary compounded nutraceutical formulation for liver support, and had their blood serum chemistries re- evaluated after at least 30 days of therapy.

Statistical analysis was applied to the data by an independent statistical consultant[4].

Results

64 dogs were included in this study; 41 were neutered males, the other 23 were spayed females. Intact individuals were neutered upon entry to the blood donor facility to facilitate their social integration into Hemopet's closed colony.

The average age of the dogs upon admission to the colony was 2.8 years (Range: 1 yr – 5yrs). The average weight of these Greyhounds was 32.5 kg (Range: 24.8 kg – 39.6 kg) The female dogs had an average weight of 29.1 kg (Range: 24.8 kg – 35.6 kg) and the male dogs an average weight of 34.3 kg (Range: 28.5 kg to 39.6 kg).

The time between the first and second blood testing occurred in the majority of Greyhounds between 5 and 15 weeks (Range: 4-35 weeks) from the date of the initial blood testing.

The changes in serum chemistry values were compared between the admission blood testing and the follow-up blood testing using paired t-tests. Hence, each dog served as his or her own control with the difference in values for each dog calculated and then averaged.

Table 1.: Compilation of Blood Test Values

Value	% With Decrease	Mean Difference (1st-2nd)	St. Dev. For Difference	*p*-value
Total Protein	58%	0.22	0.54	0.0016
AST	72%	22.8	68.2	0.0097
ALT	97%	135.7	150.4	<0.0001
Alkaline Phosphatase	56%	5.0	22.4	0.0804
GGT	57%	1.06	3.0	0.0072
Creatinine	27%	-0.02	0.23	0.4424
Na/K	56%	0.32	3.1	0.4079

ALT as well as Total Protein, AST, and GGT showed significant decreases from the first to the second blood sampling. Creatinine, alkaline phosphatase and Na/K also demonstrated decreases from the first to the second sampling time, but they were not statistically significant.

Of the 72% of Greyhounds who had improved AST concentrations, the average decrease was 36.2 IU/L (std. dev. = 76.4 IU/L, range 1 – 445 IU/L and median decrease of 13 IU/L) (Lab Reference Norms 15-66) from the first visit to the second visit of their AST. For in this cohort who did not improve, the average increase in values was 12.2 IU/L with a maximum increase of 33 IU/L.

Of the group consisting of the 97% of Greyhounds that showed an improvement in ALT, the average decrease from the first to second visit was 142.2 IU/L (std. dev. = 148.3 IU/L, range 6 – 850 IU/L and median decrease 100 IU/L) (Lab Reference Norms: 12-118) (see Figure 1 for all subjects).

Of the 56% of Greyhounds who had a decrease in alkaline phosphatase concentration the averaged decrease between the first and second time points was 17.8 IU/L (St. Dev. = 19.9 IU/L, Range: 1-105 IU/L, and median improvement = 12 IU/L) (Lab Reference Norms: 5-131). These values were all within normal limits established by the reference laboratory, Antech Diagnostics™ .

Of the 57% of these Greyhounds who had a decrease in GGT concentration the averaged improvement from the first to the second sampling time was 3.2 IU/L (St. Dev. = 1.8 IU/L, Range: 1-8 IU/L, and median improvement = 3 IU/L) (Lab Reference Norms: 1-12). In the dogs that did not improve the average increase in GGT concentration was 2.6 IU/L (Range 1-7).

58% of Greyhounds had a decrease in Total Protein that averaged of 0.53 mg/dl from the first to the second sampling time; both sets of values were within normal limits.

There was little variation in total bilirubin values from the first to the second sampling. Creatinine had a little more variation than total bilirubin values from the first and the second sampling, but the variation was also numerically quite small. The variation between the first and second sampling was 0.04 mg/dl which is not significantly different than zero.

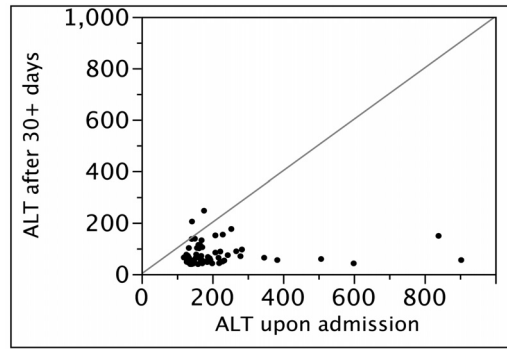

Figure 1: ALT concentrations compared from the first to the second blood tests

Discussion

This study demonstrates that Hepato Support™ was effective in resolving naturally occurring elevated liver enzyme test concentrations in the retired racing Greyhound (RRG) dogs in this closed blood donor colony. Elevated liver enzyme concentrations are not common in the RRG, but can be a sign of endogenous liver disease, or toxin exposure and subsequent hepatocellular damage. Other than blood chemistry analyses, no other attempt to further diagnose the cause of the elevated ALTs in this donor colony was made since the animals were outwardly in good health as determined by thorough physical examination by the professional veterinary staff on site, and elevated liver values normalized with such a high rate of efficacy following the nutraceutical treatment.

Racing Greyhounds are subjected to numerous stressors during their tenure at the track that can cause elevated liver enzyme concentrations. These stressors include, but are not limited to diet (1) (2), chemical exposures, vaccines, heartworm and external parasite preventatives and treatments, social anxiety from confinement, and the high energy demand of training and racing. In spite of these stressors, most Greyhounds do not develop elevated liver enzymes concentrations.

At Hemopet, a review of 2 years of blood tests from January 1, 2010 to December 27, 2011 found that out of a total of 1025 Greyhounds, only 2.4% were found to have elevated ALT concentrations. A 1997 review of 34 randomly selected healthy Greyhounds from the colony census of 75 dogs at that time revealed the average ALT concentration to be 58+/-30 IU/L, with no clinicopathologic evidence for liver disease in this cohort.

A recent (2007) published survey of the health of retired racing greyhounds found that of the 747 Greyhounds included in the survey, there was an incidence of 2.5% (1.4-3.7 CI) of liver dysfunction in this large study group. This compares favorably with the incidence of liver enzyme elevation in the group of Greyhounds studied at Hemopet. (16)

In a study of the clinicopathologic differences between Greyhounds and the non-sighthound breeds, it was noted that Greyhounds have acquired unique physiological adaptations that separate them from the other breeds, and that reference intervals in RRG differ from those in other breeds. Differences noted in this study include: higher RBC mass, creatinine concentration, glomerular filtration rate, activities of hepatic enzymes, concentration of cardiac troponin, as well as lower WBC, neutrophil, and platelet counts, thromboelastographic values, and concentrations of serum haptoglobin, total globulins, and T4. (3) These finding parallel those seen at Hemopet since its inception in 1991 (from the company's literature)

An earlier study of a large sample of 499 non-racing healthy Greyhounds in the UK determined that the serum biochemistry reference intervals in this breed to be comparable to the study cited above. (4)

The sample population at Hemopet is much more homogenous than either of the populations evaluated in each of these studies cited above. The facility is a closed colony, with the residents having been pre-selected for the same blood group genetics (blood type DEA 4), as well as their acquisition from the same greyhound training kennels in cooperation with retired racer rescue groups in Arizona, Texas and Oklahoma. Additionally, all dogs in this colony receive the same high quality diet and are housed, exercised and socialized uniformly.

Silymarin is the flavolignan complex purified from the seed of the milk thistle plant (*Silybum marianum*) that has been found historically to be effective in the treatment of liver disease, having been mentioned in herbal texts in Europe since the 16th and 17th centuries. (5) Milk thistle's early "folk" medicine application was for mushroom poisoning, especially by *Amanita* spp.

Silymarin, as a flavonoid, possesses antioxidant properties that historically has been used in the treatment of a number of human diseases, including liver disease, cancer and diabetes mellitus. One proposed mechanism of action of silymarin is to increase the intracellular glutathione content. A recent study in cats at Colorado State University found that one of the flavolignan components of silymarin, silibinin, appeared to increase neutrophil glutathione content and phagocytic function, both of which could be helpful to cats that suffer from conditions associated with increased oxidative stress. (18)

Numerous studies in laboratory animals and humans have determined that silymarin can benefit the liver. However, very few studies exist in the veterinary literature, and only a handful have been performed in the canine species. (6) (7) (11) (12) (13) (14) A thorough literature review did not retrieve any published studies of the effect of silymarin in the Greyhound with elevated liver enzyme concentrations.

Silymarin has been found to possess the following attributes (8) (17):

1. Antioxidant; scavenges ROS and protects against glutathione depletion
2. Inhibits hepatocyte membrane lipid peroxidation
3. Antifibrotic
4. Anti-inflammatory
5. Increases hepatic protein synthesis
6. Increases rate of hepatocyte regeneration
7. Choleretic; thins the bile, stimulates contraction of gall bladder
8. Protects DNA by reducing lipoxygenase, hydrogen peroxide, and superoxide radicals
9. Suppresses transcription factor (NF)-kappaB
10. Chelates iron which helps to spare glutathione with iron overload
11. Stabilizes mast cells
12. Slows calcium metabolism
13. Decreases activity of promoters of cancer growth.

The nutraceutical formula used in this study, Hepato Support™ , contains silymarin complexed with hepatotrophic nutraceuticals to facilitate normalization of liver function. The addition of these nutrients improves the efficacy of the silymarin alone by supporting the metabolic, antioxidant, and detoxification functions of the liver. (9)

Published dosages for silymarin range from 10-20 mg/kg divided daily, suggested for most clinical conditions. Silymarin can be given safely at dosages as high as 50 mg/kg/day to treat progressed hepatic pathology. (5) (10) These are doses that have been derived in experimental animals or empirically in human patients. There are no studies that were designed to establish effective dosing ranges for silymarin in dogs, much less in Greyhounds.

In this study, a set dosage of 80 mg of silymarin SID-BID was administered to these 64 participants with elevated liver enzyme concentrations. The average weight of the participants in this study was 32.5 kg, from which the average dosage administered in this study can be calculated to be 2.5-5.0 mg/kg/day.

Greyhounds have been found to have reduced liver detoxification capabilities, and sighthounds in general are known to have different pharmacokinetics with respect to drug disposition. (15) The professional veterinary staff at Hemopet generally reduce or modify the dosage of each pharmaceutical administered, including nutraceuticals such as silymarin to account for this pharmacokinetic difference in the Greyhound.

Conclusion

In this evaluation of 64 retired racing Greyhounds with elevated ALT concentrations, the administration of the nutraceutical liver support formula, Hepato Support™ was effective (overall response rate of 97%), in addition to having been well-tolerated by the study participants with no adverse reactions noted in these dogs. This ret-

rospective case series study suggests that Hepato Support™ is effective in normalizing elevated ALT concentrations in retired racing Greyhounds that have been recently adopted from the track. Prospective and controlled studies are indicated to better evaluate the clinical role of Hepato Support™ for the retired racing Greyhound and for any dog presenting with elevated alanine aminotransferase (ALT) concentrations.

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1. Hemopet; 11561 Salinaz Avenue, Garden Grove, CA 92843.
2. Hepato Support™ ; www.RxVitamins.com/pets
3. Antech Diagnostics; 17672-A Cowen Avenue, Irvine, CA 92614.
4. Karen Copeland, PhD.; www.Boulderstats.com