

Incidence, Timing, and Risk Factors of Azathioprine Hepatotoxicosis in Dogs

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Background: The use of azathioprine (AZA) in dogs is limited by the development of hepatotoxicosis and cytopenias.

Hypothesis and Objectives: To characterize the observed incidence, timing, and risk factors for AZA hepatotoxicosis in dogs treated clinically, and to determine the relationship between the development of hepatotoxicosis and cytopenias.

Animals: Fifty-two dogs treated with AZA with clinical and biochemical follow-up, with a subset of 34 dogs available for determination of changes in liver enzyme activities in serum.

Methods: Retrospective medical record review, from January 2009 through December 2013.

Results: Hepatotoxicosis (as defined by a >2-fold increase in serum ALT) was observed in 5 of 34 dogs (15%) within a median onset of 14 days (range, 13–22 days). Dogs had a median 9-fold increase in ALT and 8-fold increase in ALP, which stabilized or resolved with drug discontinuation or dose reduction. German shepherds were significantly over-represented (3 of 5 dogs with hepatotoxicosis; $P = .0017$). Thrombocytopenia or neutropenia were seen in 4 of 48 dogs with CBC follow-up (8% of dogs), but occurred significantly later in treatment (median onset, 53 days; range 45–196 days) compared to hepatotoxicosis ($P = .016$).

Conclusions and Clinical Importance: These results support the routine monitoring of liver enzymes during the first 1–4 weeks of AZA treatment in dogs, with continued monitoring of the CBC. Additional studies are warranted to characterize the apparently higher risk of AZA hepatotoxicosis in German shepherds.

Key words: ALT; German shepherd; Liver toxicosis; Thiopurines.

Introduction

Azathioprine (AZA) is a purine analog that is used as an immunosuppressive drug in both human and veterinary medicine. AZA is commonly used in dogs to treat immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, and other immune-mediated diseases.¹

The use of AZA is limited by the risk of hepatotoxicosis, which can be either idiosyncratic or dose-dependent in humans.² Idiosyncratic hepatotoxicosis from AZA is relatively rare, is typically seen within the first four weeks of administration, and can also include rash, fever, arthralgia, or myalgia.³ In contrast, dose-dependent AZA hepatotoxicosis occurs in 4–24% of human patients and is defined by an alanine aminotransferase (ALT) activity greater than 2-fold the upper limit of the reference range in patients with previously normal liver values.^{4–8} Dose-dependent AZA hepatotoxicosis typically occurs in human patients within 2–8 weeks of starting the drug,⁴ although some patients are affected later in treatment.⁵ Toxicosis has been attributed to oxidative stress and mitochondrial damage secondary to AZA bioactivation.^{9–11} In rodent models, glutathione precursors are protective.¹¹

AZA hepatotoxicosis is also observed in dogs in which experimentally, relatively high chronic oral

Abbreviations:

| | |
|-------|----------------------------------|
| 6-MMP | 6-methyl mercaptopurine |
| 6-TGN | 6-thioguanine nucleotides |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferases |
| AZA | azathioprine |
| GST | glutathione-S-transferase |
| IBD | inflammatory bowel disease |
| IMHA | immune-mediated hemolytic anemia |
| IMPA | immune-mediated polyarthropathy |
| ITP | immune-mediated thrombocytopenia |
| SAM-e | S-adenosylmethionine |
| TMPT | thiopurine methyltransferase |

dosages lead to increased transaminase activities and focal hepatic necrosis.^{12,13} In 40 dogs treated clinically for meningoencephalitis, increases in serum ALT were reported in 1 dog, but most dogs did not undergo liver enzyme monitoring.¹⁴ In dogs that were monitored while receiving AZA monotherapy for atopic dermatitis, greater than 2-fold increases in ALT were noted in 9 of 12 dogs, and 3 of these dogs had clinical signs consistent with hepatotoxicosis (vomiting, lethargy, and in one case, jaundice) that led to AZA discontinuation.¹⁵ However, aside from these small studies, AZA hepatotoxicosis in dogs is not well characterized. The primary aim of this retrospective study, therefore, was to characterize the observed incidence, timing, risk factors, and outcomes for hepatotoxicosis in dogs treated with AZA for various clinical indications. On the basis of findings in humans and our clinical experience, we hypothesized that the incidence of hepatotoxicosis (using the definition of a new increase in ALT activity greater than 2-fold the upper limit of the reference range),^{4–8} in dogs treated with AZA would be between 10 and 25%. We also hypothesized that these enzyme changes would be subclinical in most dogs. A secondary aim was to

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determine the relationship between the development of hepatotoxicosis and either thrombocytopenia or neutropenia in dogs treated with AZA.

Materials and Methods

Case Selection

Medical records of dogs that were prescribed AZA at the University of Wisconsin Veterinary Medical Teaching Hospital (UW Veterinary Care) were identified by computerized medical record review for the 5-year time period between January 2009 and December 2013. To be eligible for inclusion, dogs needed to be treated with AZA at standard therapeutic dosages for any clinical indication, and be followed up for at least 2 months or until an adverse event.

Medical Records Review

Data that were extracted from the medical records included signalment and body weight, underlying indication for AZA, baseline CBC and serum activities of liver-derived enzymes (minimally ALT), start date and daily dose of AZA along with dose adjustments over time, status of glucocorticoid dosing when AZA was started, administration of other drugs while on AZA, follow-up serum activities for ALT and a complete blood count (CBC) during the first 2 months of treatment (and again between 2 and 6 months of treatment when available). In addition, observation of clinical adverse effects possibly attributable to AZA, development of concurrent cytopenias, actions taken if dogs showed clinical or biochemical evidence of hepatotoxicosis, and response to either drug discontinuation, dose reductions, or addition of SAMe containing supplements were recorded. Hepatotoxicosis was defined as a new increase in serum ALT activity greater than 2-fold the upper limit of the reference range⁴⁻⁸ in dogs with previously normal liver values, with no other drug additions or dosage increases. Dogs that were being treated with glucocorticoids were only eligible for inclusion if they were on a stable or decreasing dosage of glucocorticoids during the observation period.

Exclusion Criteria

Primary care veterinarians were contacted to obtain additional follow-up data as needed. Dogs were excluded from the study if they had inadequate follow-up to determine clinical or biochemical response during at least one examination in the first 2 months of treatment. Dogs that were newly started on glucocorticoids or had an increase in glucocorticoid dosage in the 2 weeks preceding baseline blood work were excluded from evaluation of changes in serum ALT and ALP during the first 2 months of AZA treatment.

Statistical Analyses

Risk factors for hepatotoxicosis were determined using a Mann-Whitney *U*-test for continuous variables (age and dosage) and by a Fisher's exact test with odds ratios for categorical variables (breed, development of concurrent thrombocytopenia, or neutropenia). All analyses were performed using a commercial software package.^a

Results

Sixty-seven medical records of dogs prescribed AZA at UW Veterinary Care from 2009 through 2013 were screened for the study. Of these, 15 records were censored for inadequate follow-up after AZA administration (*n* = 5 dogs), euthanasia or death because of

severity of the underlying disease (IMHA, protein-losing enteropathy) or a concurrent condition (gastric dilation and volvulus, aspiration) before response to AZA could be assessed (*n* = 7 dogs), or lack of administration of the prescription (*n* = 3). Therefore, 52 dogs were included in outcome analyses. These dogs had been prescribed generic AZA from one or more manufacturers over the period of observation.

Breeds that were represented more than once were Labrador retrievers (*n* = 7), German shepherds (*n* = 4), Cocker spaniels and Cairn terriers (*n* = 3 each), and boxers and Maltese (*n* = 2 each; Table 1), with a median age of 7 years at the time of starting AZA. The median starting dosage of AZA was 1.9 mg/kg/day (range, 0.5–4.8 mg/kg, with a median duration of 1.7 months (range, 2 weeks to >1 year). The most common underlying conditions were inflammatory central nervous system (CNS) diseases, immune-mediated hemolytic anemia (IMHA), and immune-mediated thrombocytopenia (ITP; Table 1).

For evaluation of hepatotoxicosis, 17 of the 52 dogs had prednisone started or dose escalated within the 2 weeks before starting AZA, or had prednisone dosage increases during the observation period after starting AZA. An additional dog developed evidence of pneumonia and possible sepsis that made interpretation of posttreatment liver enzymes difficult. These 18 dogs were excluded in analyses of changes in serum-derived liver enzyme activities during the initial 2 months of

Table 1. Demographic data for 52 dogs treated with azathioprine (AZA), with clinical follow-up, from 2009 to 2013. Data are reported as medians with ranges.

| | |
|-------------------------------|--|
| Age | 7.0 years (0.9–14.0) |
| Sex | FS <i>n</i> = 23 FI <i>n</i> = 2 MN <i>n</i> = 24 MI <i>n</i> = 3 |
| Breed | Labrador retriever <i>n</i> = 7 German shepherd <i>n</i> = 4 Cocker spaniel <i>n</i> = 3 Cairn Terrier <i>n</i> = 3 Boxer <i>n</i> = 2 Maltese <i>n</i> = 2 Other individual purebreds <i>n</i> = 23 Mixed breeds <i>n</i> = 8 |
| Underlying Diagnosis | Inflammatory CNS <i>n</i> = 11 IMHA <i>n</i> = 8 ITP <i>n</i> = 7 IMPA <i>n</i> = 5 IBD/protein losing enteropathy/ lymphangiectasia <i>n</i> = 5 Pemphigus foliaceus <i>n</i> = 4 Evans syndrome <i>n</i> = 2 Lupus <i>n</i> = 2 Other immune disorders <i>n</i> = 8 |
| AZA dose (mg/kg) | 1.9 (0.5–4.8) ^a |
| AZA dose (mg/M ²) | 52.6 (16.3–107.5) |
| AZA duration (months) | 1.7 (0.25–12+) |

^aThe outlier dose of 4.8 mg/kg was prescribed to a toy breed with meningoencephalitis of unknown etiology. This dog did not have evidence of liver or bone marrow toxicity.

AZA treatment. Of the remaining 34 dogs, 5 (15%) developed biochemical evidence of hepatotoxicosis during the first 2 months of treatment (Table 2). The pattern of hepatotoxicosis was mixed (both hepatocellular and cholestatic), with a median increase in ALT of 9.0-fold (range, 2.9–23) among hepatotoxic dogs, and a median increase in ALP (serum alkaline phosphatase) of 8.0-fold (range, 1.1–19), compared to pre-AZA values. Dogs characterized as having hepatotoxicosis from AZA were either not on glucocorticoids ($n = 1$), were on a stable dosage of glucocorticoids for 3 weeks before baseline evaluation and throughout observation ($n = 1$), or were on a tapering glucocorticoid dosage during the observation period ($n = 3$). The median time to identification of hepatotoxicosis was 14 days (range, from 13–22 days). One each of the 5 dogs developed new hypoalbuminemia (2.1 g/dL) or mild hypocholesterolemia (137 mg/dL), while bilirubin, BUN, and glucose remained normal (Table 2). Only 1 of 5 dogs had clinical signs of illness at the time of identification of biochemical hepatotoxicosis (anorexia and diarrhea in a dog with underlying protein-losing enteropathy), and no dog showed evidence of jaundice, encephalopathy, or ascites. None of the other dogs showed new biochemical evidence of hepatotoxicosis between 2 and 6 months after starting the drug, although liver panels were available for only 24 dogs during this period.

There were no detectable differences between affected and unaffected dogs with regard to age at AZA administration (median 7.0 versus 7.0 years, $P = .88$) or AZA dosage, either expressed as mg/kg (median 1.7 versus 1.9 mg/kg/day; $P = .96$) or mg/M^2 (52.6 versus 49.0 mg/M^2 /day; $P = .51$). However, 3 of 5 dogs affected by hepatotoxicosis were German shepherds (60%), compared to none of the 29 dogs without hepatotoxicosis OR = 82.6, 95% CI, 3.2–2,094; $P = .0017$. The underlying diseases in the affected German shepherds were inflammatory CNS disease, ITP, and systemic lupus erythematosus.

AZA was dose-modified for all dogs that developed hepatotoxicosis. Two of the 5 dogs had AZA discontin-

ued. Unfortunately, only one of these dogs had serum liver enzymes re-evaluated, which showed a decrease in ALT from >2,000 IU/L to 400 IU/L. This dog was also treated with a SAM-e supplement^b during this period. The other 3 dogs had a 50% dose reduction in AZA; in all of these dogs, ALT remained stable or was decreased at the next recheck with no development of clinical signs.

As a secondary aim, the development of thrombocytopenia or neutropenia was also evaluated in the same population of 52 dogs. Of the 48 dogs that had CBCs measured before and after AZA, 4 (8.3%) developed cytopenias: 2 dogs with neutropenia (2,500 and 2,640/ μL), 1 dog with thrombocytopenia (142,000 μL), and one dog with both (2,088 neutrophils/ μL and 12,000 platelets/ μL). We did not track drug-induced anemia separately because of the substantial number of patients with pre-existing IMHA, ITP, or Evans syndrome; however, none of the dogs with thrombocytopenia or neutropenia in this population had evidence of aplastic anemia (hematocrit values were 32–45%).

None of the dogs with thrombocytopenia or neutropenia were the same dogs that developed hepatotoxicosis, and evidence of bone marrow suppression developed a median of 53 days (range 45–196 days) after starting AZA, which was significantly later than the time to hepatotoxicosis ($P = .016$). None of the 4 dogs with evidence of neutropenia or thrombocytopenia had clinical evidence of fever or petechiae. The dosage of AZA was reduced by 50–75% in all dogs with cytopenias; AZA was ultimately discontinued in 2 of these dogs due to persistent neutropenia over 3–6 weeks.

Discussion

In the dogs of this study, the observed frequency of hepatotoxicosis (as defined by a greater than 2-fold increase in ALT activity) was 15%. This should be considered an estimate only, since many treated dogs were excluded from analyses because of concurrent conditions that would affect serum ALT independent of AZA administration. The pattern of hepatotoxicosis was mixed (both hepatocellular and cholestatic) in all dogs, and despite marked increases in serum ALT in some dogs, most were without clinical signs. Although it was not noted in the dogs of this study, jaundice can occur in dogs treated with AZA monotherapy.¹⁵

In humans, AZA hepatotoxicosis can present as a hepatocellular, cholestatic, or mixed pattern, and is also typically asymptomatic.^{4,5,16,17} The incidence of hepatotoxicosis in humans depends on the patient population, and ranges from 4 to 24%,^{4,5,7,18} with only 3–4% requiring AZA discontinuation because of more severe manifestations of hepatotoxicosis.^{5,7} Liver failure from AZA is uncommon in human patients,¹⁹ and in fact, AZA is considered a standard first line agent to treat autoimmune hepatitis in people.²⁰

Azathioprine led to increases in liver-derived serum transaminases and focal hepatic necrosis in dogs dosed experimentally with 1–4 mg/kg/day for 1–6 months.^{12,13} In our study, dosage was not a detectable risk factor

Table 2. Serum biochemical variables in 5 dogs that developed evidence of hepatotoxicosis during azathioprine (AZA) treatment. Data are reported as medians and ranges.

| Biochemical variable Measurement (Reference Interval) | Before AZA | After AZA |
|---|-----------------------------|----------------------------|
| ALT (14–87 IU/L) | 71 (49–150) | 782 (252–>2,000) |
| ALP (20–157 IU/L) | 142 (109–1364) | 1253 (175–>3,000) |
| Bilirubin (0.1–0.8 mg/dL) | 0.1 (<0.1–0.2) | 0.1 (<0.1–0.5) |
| Albumin (2.3–3.9 g/dL) | 3.3 (<1.0–3.8) ^a | 3.1 (1.6–3.5) ^a |
| Blood urea nitrogen (BUN; 7–32 mg/dL) | 17 (15–21) | 15 (10–39) |
| Cholesterol (149–319 mg/dL) | 180 (154–205) | 265 (137–482) |
| Glucose (67–132 mg/dL) | 94 (90–98) | 98 (85–106) |

^aThe dog with a serum albumin of <1.0 g/dL before treatment and 1.6 g/dL after treatment had protein-losing enteropathy.

for hepatotoxicosis, but the number of affected dogs was small. Unexpectedly, German shepherd dogs were significantly over-represented in the hepatotoxic group. This could reflect differences in AZA bioactivation or detoxification in the German shepherd breed, and this observation deserves further investigation. AZA hepatotoxicosis has been attributed to the accumulation of 6-MMP (6-methyl mercaptopurine) and its nucleotide metabolites following AZA administration.^{6,18} These metabolites are generated by the sequential actions of glutathione-S-transferases (GSTs) and thiopurine (S)-methyltransferase (TMPT).^{2,4,21} High activity polymorphisms in the TPMT and GST pathways increase the risk of hepatotoxicosis in humans, because of higher resulting concentrations of 6-MMP.^{2,21} These pathways merit further characterization in dogs with AZA hepatotoxicosis, particularly German shepherd dogs.

The dogs with hepatotoxicosis in our study had new increases in ALT and ALP documented after 13–22 days of AZA treatment, with a median onset of 2 weeks. No dogs were noted to develop hepatotoxicosis after 8 weeks of treatment, but serum liver enzyme monitoring was less intensive during this period, so delayed subclinical toxicosis could not be ruled out. In dogs with atopic dermatitis given AZA, increases in serum ALT were observed between 2 and 4 weeks of treatment.¹⁵ In human patients, increases in transaminases are typically noted between 2 and 8 weeks of starting AZA, although some patients can be affected after 12–18 weeks.^{4,5} Therapeutic drug monitoring for 6-MMP metabolites, along with serum liver enzyme activities, has been recommended at 1 and 4 weeks after beginning AZA in human patients.²² In the absence of clinically available assays for AZA metabolites in dogs, our results support the monitoring of serum ALT and ALP on one or more occasions between 1 and 4 weeks after starting AZA in dogs.

It is important to note that although nearly 15% of treated dogs had increases in ALT that met the traditional definition of AZA hepatotoxicosis in humans, dogs were typically without clinical signs, and no dogs had evidence of overt liver failure. Some hepatologists recommend a stricter criterion of 5-fold or more increases in serum ALT activities to define clinically significant drug-induced liver injury in humans.²³ In our study, 4 of the 5 affected dogs had serum ALT activities greater than 5-fold. The dosage of AZA was adjusted in all 5 affected dogs, so it is unclear whether clinically important hepatic dysfunction would have developed if the dosage had not been changed. Physicians manage AZA-induced hepatotoxicosis with close monitoring, dosage reductions, or drug discontinuation depending on the severity of increases in serum liver enzyme activities. For example, for transaminases that are <5-fold increased, AZA is continued and liver enzymes are monitored; for transaminases >5-fold increased, the AZA dosage is decreased by 50% with monitoring; and if hyperbilirubinemia develops, AZA is discontinued.⁷

AZA hepatotoxicosis is exacerbated experimentally by glutathione depletion, and both N-acetylcysteine and vitamin E analogs protect against AZA-induced liver

damage.^{9,11} Only one dog in our hepatotoxic group was treated with a SAM-e supplement as a glutathione precursor, so we could not draw any conclusions about efficacy. A prospective study is needed to determine whether SAM-e supplementation can decrease the incidence of AZA hepatotoxicosis, as has been shown for CCNU hepatotoxicosis in dogs.²⁴

Evidence of bone marrow toxicosis, including neutropenia, thrombocytopenia, or both, was also evaluated in our study. Although bone marrow suppression is a commonly listed adverse effect of AZA, no prevalence studies have been published in dogs. Cytopenias (neutropenia and thrombocytopenia, sometimes accompanied by non-regenerative anemia) are noted between 4 and 16 weeks after starting AZA.^{25,26} In this retrospective study, we found an 8% observed frequency for thrombocytopenia, neutropenia, or both, with a median onset of 53 days. Although our case numbers were very small, our results are comparable to what is reported in human patients, in which 3–12% develop clinically relevant cytopenias with a median onset of 2–3 months after starting AZA.^{8,27}

In our study, fewer dogs treated with AZA were observed to develop cytopenias compared to those that developed hepatotoxicosis, although numbers were too small for statistical comparisons. In addition, cytopenias developed significantly later than did hepatotoxicosis (53 versus 14 days), and no individual dogs developed both toxicoses. These findings suggest different risk factors for AZA bone marrow and liver toxicoses in dogs, and are consistent with data in humans that implicate different AZA metabolites in the two toxicities. While hepatotoxicosis is related to 6-MMP nucleotides, cytopenias are associated with high concentrations of the pharmacologically active 6-thioguanine nucleotide (6-TGN) metabolites.² In fact, the efficacy of AZA can be correlated with a high 6-TGN: 6-MMP ratio.¹⁸ In contrast to hepatotoxicosis, bone marrow suppression from AZA in humans is associated with low TMPT activities, which leads to metabolic shunting away from 6-MMP and overproduction of 6-TGNs.² The risk factors for bone marrow toxicosis from AZA have not been determined in dogs, but low TMPT activity might not be a major factor in this species.²⁸

There are several study limitations that should be considered when interpreting our data. Since the study was retrospective, we did not have biochemical data on dogs at consistent time points. Most dogs had no clinical signs and dogs were not sampled weekly, so the actual time of onset of hepatotoxicosis could not be precisely defined. In addition, dogs with increased liver enzymes at recheck appointments were not routinely evaluated with abdominal ultrasound or bile culture to look for new but unrelated hepatobiliary disease. Finally, the number of affected dogs was relatively small. Therefore, these frequency and onset data should be considered estimates, and could provide the basis for a larger, prospective study with standardized follow-up criteria.

Another potential confounding factor in our study is the concurrent administration of glucocorticoids in many of the dogs. While this is a common scenario in

dogs treated with AZA, it does confound liver enzyme analyses. We attempted to avoid this by only including dogs in serum liver enzyme analyses that were on stable or decreasing dosages of prednisone during the AZA observation period. However, the true incidence of AZA hepatotoxicosis should ideally be evaluated in dogs treated with AZA monotherapy.

In summary, we found biochemical evidence of hepatotoxicosis in 5 of 34 (15%) of dogs treated with AZA, within a median onset of 2 weeks after starting the drug, and typically without clinical signs. German shepherds were over-represented, and might be predisposed as a breed to AZA hepatotoxicosis. Hepatotoxicosis occurs earlier than bone marrow suppression in dogs, and might involve different risk factors. The results of this study support the routine monitoring of liver enzymes on one or more occasions between 1 and 4 weeks of AZA treatment in dogs, with chronic monitoring of the CBC. Additional studies are warranted to evaluate the efficacy of glutathione precursors in the prevention of AZA hepatotoxicosis.

Footnotes

^a Prism 4.0; GraphPad Software Inc, La Jolla, CA

^b Denamarin; Nutramax Laboratories Veterinary Sciences, Inc, Lancaster, SC

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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