Drug-Induced Liver Injury

Jennifer M. Reinhart, DVM, PhD

KEYWORDS

• Hepatotoxicity • Drug-associated liver disease • Adverse drug reaction
• Idiosyncratic

KEY POINTS

- Drug-induced liver injury (DILI) is underrecognized in small animals and requires a thorough clinical history to make a diagnosis.
- DILI can be intrinsic, which is generally dose-dependent, or idiosyncratic, which occurs at normal doses and affects only a small proportion of individuals.
- Many idiosyncratic DILIs are thought to be immune-mediated and may have genetic predispositions.
- Diagnosis of DILI remains dependent on identifying consistent clinical features of the individual drug toxicity and clinical improvement after drug discontinuation.

INTRODUCTION

Drug-induced liver injury (DILI) is an underrecognized cause of hepatic disease in small animal medicine. To correctly identify cases, the clinician must have a high level of suspicion for drug-related illness. This requires a thorough drug history including supplements and herbal preparations as well as assessment for possible inadvertent exposure. Diagnosis also requires an understanding of the hepatotoxic potential of various drugs; the incidence of DILI for most individual drugs is low, which may falsely decrease the index of suspicion. A final barrier to identification is that DILI has no pathognomonic findings to differentiate it from other causes of liver injury and presents with a wide range of clinical and histopathologic phenotypes. Thus, diagnosis must rely on ruling out other hepatic diseases, a temporal association with drug exposure, and appropriate response to dechallenge. Although DILI typically resolves within a few weeks of discontinuing the offending drug, it can have severe consequences if left untreated.[1] In humans, DILI is the leading cause of acute liver failure with transplant-free survival rates of 22% to 75%.[2] Thus, by improving our understanding and awareness of this disease, we can increase early identification of DILI in veterinary patients.

Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, 1008 West Hazelwood Drive, Urbana, IL 61802, USA
E-mail address: jreinha2@illinois.edu

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Abbreviations	
DILI	drug-induced liver injury
NAPQI	N-acetyl-p-benzoquinone imine
NSAIDs	nonsteroidal anti-inflammatory drugs
PTPN22	protein tyrosine phosphatase nonreceptor type 22
RUCAM	Roussel Uclaf Causality Assessment Method
TPMT	thiopurine-methyltransferase
ULN	upper limits of normal

CLASSIFICATION AND PATHOGENESIS

DILI is divided into 2 types based on clinical features and pathogenesis: intrinsic and idiosyncratic (Table 1). Intrinsic DILI is a dose-dependent and time-dependent toxicity in which the parent drug or a metabolite causes direct damage to hepatocytes, impairing function and leading to cell death. It is dose-dependent in that the risk of toxicity increases with dose, and most members of an exposed population will demonstrate hepatotoxicity given a sufficient dose. Clinical signs of intrinsic DILI usually occur soon after exposure (days) making identification of toxicity relatively easy, as long as the exposure is identified. In many situations, therapy with the offending drug can be safely reinstituted at a lower dose once the hepatic damage has resolved. Therapeutic drug monitoring may also be useful in predicting and preventing these reactions for drugs with a narrow therapeutic index.

The prototypic intrinsic DILI is acetaminophen toxicity. Acetaminophen is metabolized by the cytochrome P450 enzyme system to N-acetyl-p-benzoquinone imine (NAPQI), an oxidative metabolite that depletes intracellular glutathione and causes centrilobular hepatic necrosis. More recent studies have identified the activation of procell death signaling via NAPQI binding mitochondrial proteins as well as the initiation of adaptive cellular defense mechanisms. Therefore, intrinsic DILI is more than a simple, passive toxicity but involves an active cellular response.[3]

In contrast to the intrinsic form, idiosyncratic DILI is unpredictable and more difficult to diagnose because it only occurs in a small proportion of exposed individuals. Idiosyncratic DILI generally exhibits a latency period, which may be anywhere from a few days to months between drug exposure and development of clinical signs. Thus, it is possible that therapy with offending medication may have been discontinued for a

Table 1
Characteristics of intrinsic and idiosyncratic drug-induced liver injury

Characteristic	Intrinsic	Idiosyncratic
Predictable	Yes, therapeutic drug monitoring may be useful	No
Dose-dependent	Yes	No, but threshold exposure may be required
Onset of clinical signs	Short (days)	Long (days to months)
Management	Dose reduction (for subclinical disease), drug holiday with reinstitution at a lower dose (for clinical disease)	Permanent drug discontinuation
Mechanism	Direct damage from parent drug or metabolite	Direct damage from metabolite (usually) and/or immune-mediated damage

significant period prior to illness, emphasizing the need for a thorough drug history. Although idiosyncratic DILI is classically considered dose-independent, studies in people have demonstrated that drugs dosed above 50 mg per day have higher rates of idiosyncratic DILI than those dosed below this cutoff.[4] Furthermore, higher daily dosages lead to shorter latencies and higher mortality rates when idiosyncratic DILI does occur.[5] These results have been interpreted as a possible minimum threshold of drug exposure required to trigger the reaction rather than true dose-dependency. Nevertheless, because a strict dose–response relationship is not present for idiosyncratic reactions, once a patient has experienced hepatotoxicity, they should never be re-exposed to that drug because of the risk for reoccurrence.

Idiosyncratic DILI has been referred to as a drug "allergy" or hypersensitivity reaction, but this is an oversimplification, and an adaptive immune response has not been demonstrated for all drugs. Most drugs that cause idiosyncratic DILI undergo hepatic metabolism to reactive forms that covalently bind cellular proteins and cause oxidative stress. Thus, there is a shared pathogenesis between the intrinsic and idiosyncratic forms. However, in some cases of idiosyncratic DILI, that drug–protein binding forms neoantigens that elicit an immune-mediated response. Because of its location in the portal circulation, the liver has an overall tendency toward immunotolerance. In patients that develop idiosyncratic reactions, costimulatory factors including cytokines produced by the innate immune system and danger-associated molecular patterns derived from oxidative damage of drug adducts shift the adaptive milieu to immunoactivation. This results in a self-propagating cycle of immune-mediated tissue damage.[1]

Genetic factors may play an important role in the development of idiosyncratic DILI. Many human studies have identified associations between DILI and individual genetic variants in drug metabolizing enzymes and transporters.[6] Similarly, a single nucleotide polymorphism in the cytochrome b5 reductase gene has been linked to sulfonamide hypersensitivity in dogs.[7] It is presumed that these predisposing variants cause higher intrahepatic concentrations of reactive metabolites, which increase the risk for drug toxicity. Although these initial associations between idiosyncratic DILI and individual genes related to xenobiotic biotransformation appeared promising, most could not be confirmed with pharmacogenomic approaches.[8] Instead, genome-wide association studies in humans have identified polymorphisms in multiple immunologic genes as risk factors for DILI from various drugs. Most commonly implicated are human leukocyte antigens, which present antigen on the cell membrane for evaluation by the immune system. This emphasizes the role of neoantigen formation and recognition in DILI pathogenesis. Most genetic variants associated with DILI are unique to a specific drug or drug class. However, a polymorphism in the protein tyrosine phosphatase nonreceptor type 22 (PTPN22) is linked to DILI in people across multiple drugs.[9] PTPN22 is thought to participate in T-cell response regulation, so this may represent a common pathway for immune activation by drug adducts. Genes with immunologic function have not yet been investigated in DILI in dogs and cats. However, polymorphisms in the dog leukocyte antigen genes and canine PTPN22 have been associated with a variety of other immune-mediated conditions including symmetric lupoid onychodystrophy,[10] chronic enteropathy,[11] Addison's disease,[12] and atopy,[13] so a similar association with DILI appears possible.

A third category of "indirect DILI" has recently been proposed in human medicine.[1] In indirect DILI, hepatic damage results from the mechanism of action of the drug ("what the drug does") rather than an adverse reaction to the drug or its metabolite ("what the drug is"). Most drugs in this category interact with the immune system and disrupt normal immunoregulation leading to immune-mediated hepatitis. Examples include

immune-checkpoint inhibitors and protein kinase inhibitors. Few drugs in these classes currently exist in veterinary medicine. However, as new therapeutics are developed, particularly in the field of veterinary oncology, clinicians should be aware of the possibility of such reactions.

DIAGNOSIS OF DRUG-INDUCED LIVER INJURY

Clinical diagnosis of DILI can be quite difficult. Definitive diagnosis requires drug rechallenge wherein therapy with the offending drug is reinstituted and relapse of clinical signs indicates a positive response. However, drug rechallenge is impractical and ethically dubious, so we must rely upon other diagnostic methods. An understanding of typical biochemical and histologic patterns for individual drug culprits can be helpful when evaluating possible DILI cases. In human medicine, an R value is used to classify DILI, and the same approach has been recommended in veterinary texts.[14] The R value is the ratio of the -fold increase of alanine transaminase (ALT) and alkaline phosphatase (ALP) over their respective upper limits of normal (ULN):

$$R = \frac{\mathrm{ALT}/\mathrm{ULN}_{\mathrm{ALT}}}{\mathrm{ALP}/\mathrm{ULN}_{\mathrm{ALP}}}$$

An R greater than 5 is indicative of hepatocellular DILI; an R less than 2 is indicative of cholestatic DILI; and R = 2 to 5 constitutes mixed DILI. Concurrent use of drugs that cause benign increases in liver enzyme activities (eg, glucocorticoids and phenobarbital) can obscure biochemical detection of DILI and complicate the interpretation of an R value. The utility of liver biopsy for DILI diagnosis is somewhat controversial. Biopsy can rule out some hepatopathies including neoplasia and certain infections. Additionally, many offending drug toxicities have classic histologic appearances, which can help establish a causative link between exposure and disease. However, no histologic findings are pathognomonic for DILI, so other causes of the lesion must still be ruled out before a diagnosis can be made.

Routine biochemical and histologic testing can characterize damage and severity but lack specificity for diagnosing DILI (**Fig. 1**). Development of novel biomarkers for DILI is an active area of research in the field and has encompassed a wide variety of methods including pharmacogenetic markers, metabolomics screens, and drug-specific immunologic testing.[15] Unfortunately, thus far, most of these tests do not perform well enough for clinical application. Therefore, the integration of clinical

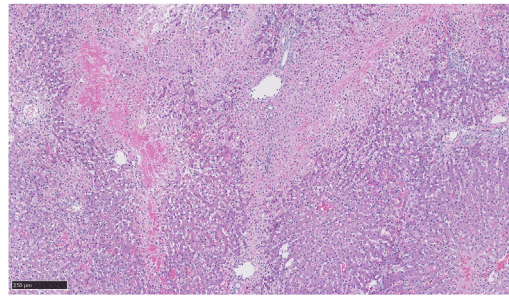

Fig. 1. Histologic image of the liver from a dog with suspected drug-induced liver injury. Multifocal to coalescing areas of coagulative to liquefactive necrosis in periportal to midzonal hepatocytes. While common in cases of acute drug-induced liver injury, they are not specific findings and can also be associated with other etiologies.

information remains the best strategy for making a DILI diagnosis. To assist with this process, several causality scoring systems have been proposed in human medicine.[16,17] The current standard for DILI diagnosis via causality assessment is the Roussel Uclaf Causality Assessment Method (RUCAM).[16] The RUCAM uses separate scoring systems for hepatocellular and cholestatic/mixed injury and divides the likelihood of DILI into excluded, unlikely, possible, probable, and highly probable. Each case is scored on the following 7 items:

- Time to onset from first exposure
- Course of ALT/ALP after cessation of drug
- Risk factors (alcohol use, age, and pregnancy status)
- Concomitant drug or herbal use
- Search for alternative causes (infectious serology/polymerase chain reaction, imaging, and concurrent disease)
- Hepatotoxic potential of the drug
- Response to unintentional re-exposure

Application of the RUCAM to veterinary patients would require modification and validation. However, it does address the major factors that should be considered when evaluating the possibility of DILI, and so could be used as a qualitative schema by veterinarians for individual patients.

HEPATOTOXICITY OF SELECT VETERINARY DRUGS

DILI is difficult to study in veterinary medicine because of underrecognition and low case numbers reported in the literature. Although a multitude of drugs are known or suspected to cause liver injury in dogs and cats (Table 2), these toxicities are often poorly characterized. There are many drugs that cause mild-to-moderate liver enzyme increases in dogs and cats but uncommonly cause clinical illness. Authors disagree about when cases should be considered true liver injury and which cases require intervention. Because of limited information in veterinary species, classification of intrinsic

Table 2
Drugs known or suspected to cause drug-induced liver injury in dogs and cats

Probable Intrinsic DILI	Probable Idiosyncratic DILI
Acetaminophen	Beta-lactam antibiotics
Amiodarone	Diazepam (cats)
Azathioprine	Felbamate (dogs)
Azole antifungals	Glucocorticoids (dogs)
Cyclosporine	Griseofulvin (cats)
Doxycycline	Imidocarb (dogs)
Glipizide (cat)	Mebendazole (dogs)
Halothane/methoxyflurane	Methimazole (cats)
Leflunomide (dog)	Mitotane (dogs)
Lomustine (dogs)	Mycophenolate
Methotrexate (dogs)	NSAIDs (dogs)
Phenobarbital (dogs)	Phenytoin (dogs)
Primidone (dogs)	Rivaroxaban (dogs)
Rifampin (dogs)	Sulfonamide antibiotics (dogs)
Stanozolol (cats)	Terbinafine (dogs)
Thiacetarsamide	Trazodone (dogs)
Toceranib	Zonisamide (dogs)

Unless otherwise indicated, toxicity may occur in both species. This list is nonexhaustive.

versus idiosyncratic DILI can be difficult. Hepatotoxicities that occur somewhat commonly or have evidence of dose-dependency are usually classified as intrinsic. However, in light of the possible need for a threshold dose in idiosyncratic toxicity, some of these classifications should be revisited.

Azathioprine

Azathioprine-associated DILI is characterized by a hepatocellular, cholestatic, or often mixed hepatic enzyme pattern with or without hyperbilirubinemia and clinical signs of hepatopathy. In dogs, it has a prevalence of 15% and is a dose-dependent reaction; dose reductions can stabilize or improve ALT and ALP activities.[18,19] Azathioprine treatment in dogs is routinely tapered from its initial starting dose of 2 mg/kg/d to 0.5 to 1 mg/kg daily or every other day over the first few weeks of treatment.[20] DILI generally occurs within the first 4 weeks of therapy so this should be a period of increased monitoring and, if DILI is detected, a rapid dose reduction should be instituted. This timeframe is in contrast to that of myelotoxicity, which is also a dose-dependent adverse effect of azathioprine, but tends to occur weeks to months into therapy.[19]

Azathioprine undergoes complex metabolism in the liver. Hepatotoxicity is thought to be mediated by the 6-methylmercaptopurine metabolite, which is generated by the enzyme thiopurine-methyltransferase (TPMT).[21] In people and dogs, TPMT activity is variable across the population, which suggests there could be genetic predispositions to azathioprine-associated DILI, but this has not been documented.[21,22] As a species, cats have extremely low TPMT activity, which might protect them from hepatoxicity, but puts them at high risk for myelotoxicity.[23] Hence, azathioprine should be used with extreme caution in cats.

Azole Antifungals

All azole antifungal drugs have the potential to cause DILI in small animals and routine biochemical monitoring is recommended. Most commonly, hepatotoxicity manifests as mild-to-moderate increases in ALT and/or ALP without clinical signs. However, severe, clinical hepatic disease with hyperbilirubinemia and hepatic dysfunction can occur. It is unclear whether these presentations represent a continuum of disease or are distinct toxicity syndromes. Azole-associated DILI is considered a dose-dependent hepatotoxicity, and this has been demonstrated for itraconazole in dogs. Legendre and colleagues[24] prospectively treated 112 dogs with blastomycosis with 5 mg/kg versus 10 mg/kg itraconazole daily. After 30 days of treatment, 60% of dogs receiving the high dose had increased ALT whereas only 12% receiving the low dose had increased ALT. Results were similar for ALP and both enzyme activities were positively correlated with serum itraconazole concentrations. Despite the difference in dose, there was no significant difference in cure (54.3% vs 53.6%) or relapse (20% vs 21.4%) rates between groups, which is why 5 mg/kg/d is the recommended starting dose for itraconazole when treating canine blastomycosis.

The mechanism of hepatotoxicity of azole antifungals is poorly understood and may depend on the individual drug. Ketoconazole-associated DILI is thought to be caused by an oxidative metabolite, N-deacetyl-ketoconazole; however, itraconazole, a parent drug, appears to be more hepatotoxic than any of its known metabolites.[25,26] Comparing between azole drugs, the imidazoles, including ketoconazole, are considered more hepatotoxic than the triazoles based on rodent and human studies.[27,28] However, a veterinary retrospective study found that ALT increases rarely occurred in dogs prescribed ketoconazole for dermatologic disease.[29] Among the triazoles, itraconazole is more commonly associated with DILI than fluconazole in people,

with liver enzyme increases occurring in 18.9% versus 10.0%, respectively. In a study of canine blastomycosis, 26% of dogs treated with itraconazole had increased ALT compared with 17% of dogs treated with fluconazole, but this difference was not statistically significant.[30] A similar trend was seen for dogs with histoplasmosis treated with itraconazole versus fluconazole.[31] Increased liver enzyme activity associated with itraconazole may improve or resolve after switching to fluconazole therapy.[31,32] Dose-reduction can also be an effective strategy, and therapeutic drug monitoring may be a useful tool for this approach.[32] The incidences of hepatotoxicity associated with voriconazole, posaconazole, and isavuconazole are unknown in dogs and cats, but DILI is a known adverse effect of all 3 drugs in people.[33–35]

Carprofen

DILI is an idiosyncratic adverse effect of nonsteroidal anti-inflammatory drugs (NSAIDs), and carprofen is the most commonly implicated. Most dogs with carprofen-associated DILI present within 5 to 30 days of drug initiation, but chronic cases (2–6 months) are reported. Clinical signs are generally nonspecific, and the biochemistry is characterized by a hepatocellular to mixed liver enzyme pattern. Hyperbilirubinemia occurs in most cases and ranges from mild to severe. Acute hepatic necrosis is the primary histologic lesion accompanied by varying degrees of neutrophilic or lymphocytic inflammation, fibrosis, and biliary hyperplasia. The prognosis for carprofen-associated DILI is generally good with approximately 80% survival in one study. In survivors, clinical signs resolve with a few days of drug discontinuation and biochemical abnormalities reach normal or near-normal values by 1 to 3 months.[36]

Despite widespread concern, carprofen-associated DILI is quite rare. In 2 large-scale studies, hepatotoxicity occurred in 2 out of 805 and 0 out of 110 dogs.[37,38] The manufacturer has estimated the incidence at 1.4 cases per 10,000 dogs. Labrador retrievers were overrepresented in the initial case series of carprofen-associated DILI (13 out of 21 dogs).[36] However, it is now believed that this may be due to the popularity of the breed in the United States and their propensity to develop osteoarthritis, rather than a true breed predisposition.

Diazepam

First reported in the 1990s, oral diazepam has been linked to acute hepatic necrosis in cats. The true incidence of this adverse drug reaction is unknown but is likely low as the toxicity is considered idiosyncratic in nature. In 2 case series, clinical signs of lethargy, ataxia, and anorexia began 5 to 13 days after the initiation of treatment.[39,40] Diazepam-associated DILI is characterized by severely increased hepatocellular enzyme activities (ALT and aspartate transaminase [AST]) with normal to modest increases in cholestatic enzymes (ALP and gamma-glutamyl transferase [GGT]) and moderate hyperbilirubinemia. Biochemical evidence of hepatic dysfunction is also routinely present and can include abnormal coagulation parameters.[40] Diazepam-associated DILI has a classic histologic appearance of severe, acute to subacute, lobular to massive hepatic necrosis with moderate to marked biliary hyperplasia.[39,40] The pathogenesis is unknown, but it has been proposed that the apparent predisposition of cats is due to their decreased glucuronidation capacity of oxidative diazepam metabolites.[41] It should be noted that DILI has only been reported with *oral* diazepam in cats and not with other routes of administration. This could represent a unique feature of diazepam biotransformation in the cat, possibly related to first-pass metabolism. Alternatively, this might simply be because diazepam is usually administered orally when repeated dosing is necessary, injectable products are reserved for single-dose rescue treatments.

Doxycycline

DILI is an underrecognized adverse effect of doxycycline in small animals. In a retrospective study of dogs treated with doxycycline, 39% and 36% developed increases in ALT and ALP, respectively.[42] The prevalence of clinical signs of hepatotoxicity was not reported but is likely much lower. Interestingly, ALP was correlated with doxycycline dose, but ALT was not, so it is unclear whether a dose-dependent mechanism is present. However, if doxycycline-associated DILI is dose-dependent, it should be noted that the median dose used in that study (16 mg/kg/d, range 5–30 mg/kg/d) was higher than what is typically used, which could have influenced the frequency of liver enzyme increases. ALT and ALP activity increases also occur in 19% and 6%, respectively, of cats receiving doxycycline and at least one case of clinical tetracycline-associated DILI has been reported in a cat.[43,44]

Lomustine

The chemotherapy agent lomustine is a major cause of veterinary DILI with 29% of treated dogs developing ALT increases at least 5 times the upper limit of the reference interval and 6% developing clinical hepatotoxicity.[45,46] DILI typically occurs after 1 to 3 doses of lomustine, and the risk increases with cumulative dose.[45] Severe ALT elevation may be preceded by more mild increases, but about half of dogs with lomustine-associated DILI develop biochemical abnormalities without warning.[45] In some cases, toxicity is delayed and clinical illness not recognized for weeks to months following treatment cessation.[46] Histologic lesions include loss of portal vein profiles, biliary atypia, and fibrosis; the severity of these lesions correlate with peak liver enzyme activities.[47] Lomustine-associated DILI is associated with decreased hepatic glutathione concentrations and supplementation with a glutathione precursor (S-adenosylmethionine/silybin) significantly reduced the prevalence, severity, and complications of hepatotoxicity in dogs.[47,48] Clinically significant lomustine hepatotoxicity is rare in cats.[49]

Methimazole

DILI is a rare adverse effect of methimazole, occurring in about 2% of cats usually within 2 months of initiating treatment. It is characterized by a hepatocellular enzyme pattern with hyperbilirubinemia and clinical signs of anorexia, vomiting, lethargy, and icterus. Clinical and biochemical changes reverse within days to weeks of drug withdrawal.[50] As with most other methimazole adverse effects, the hepatopathy is idiosyncratic, so the drug should not be reinstituted if it occurs and other treatment modalities should be pursued.

Phenobarbital

Phenobarbital-associated DILI is a rare complication of chronic therapy in dogs and must be differentiated from the benign increases in ALP that phenobarbital commonly causes. Phenobarbital hepatotoxicity typically occurs after several months to years of treatment and is characterized by a cholestatic to mixed liver enzyme pattern with or without hyperbilirubinemia and hypoalbuminemia.[51] Histologically, the toxicity is characterized by bridging fibrosis and nodular regeneration, although animals can improve clinically after the withdrawal of phenobarbital and supportive management. In one case series, 13 out of 18 dogs with phenobarbital-associated DILI had serum phenobarbital concentrations greater than 35 μg/mL, so this toxicity is considered dose-dependent.[51] Serial monitoring of liver values is recommended at least every 6 months for dogs on chronic phenobarbital treatment with subsequent hepatic function testing (eg, bile acids) indicated if toxicity is suspected.[52]

Rifampin

Due to the increasing prevalence of methicillin-resistant *Staphylococcus* organisms, there has been renewed interest in using rifampin in small animals.[53] However, rifampin is a known cause of DILI in dogs, especially at higher doses.[20] Increases in ALT and ALP are reported in 6% to 27% and 24% of dogs, respectively, and higher ALT activity was associated with an increased risk of clinical adverse effects (vomiting, anorexia, and weight loss) in one study.[54–56] Liver enzyme increases occur most commonly 3 to 4 weeks into the rifampin course, which is also when clinical signs are most likely.[54,55] This has led to the recommendation for weekly biochemical assessment with prioritization of the pretreatment and week 3 or 4 samples.[54]

Sulfonamide Antibiotics

DILI associated with sulfonamide antibiotics is part of a larger, idiosyncratic adverse drug reaction syndrome called sulfonamide hypersensitivity or "sulfa allergy." Components of sulfonamide hypersensitivity may occur alone or in combination and begin an average of 12 days (range 5–36 days) after first exposure.[57] In one study, hepatopathy was the third most common manifestation (28%), with fever (55%) and thrombocytopenia (54%) occurring more frequently.[57] Biochemically, sulfonamide antibiotic-associated DILI may present as hepatocellular or cholestatic injury or as a combination of the two. Acute hepatic necrosis is classically seen on histology, often with varying degrees of lymphoplasmacytic infiltrates.[58] Although Doberman Pinschers are overrepresented for sulfonamide hypersensitivity, their disease tends to manifest as polyarthropathy rather than as hepatotoxicity.[59,60]

The pathogenesis of sulfonamide hypersensitivity is one of the best characterized idiosyncratic drug reactions owing to its importance in human medicine (**Fig. 2**). All sulfonamide antibiotics contain an arylamine ring that, under oxidative conditions, is biotransformed to a reactive metabolite. The reactive metabolite covalently binds endogenous proteins forming neoantigens, which elicits an immune response.[61] The immunologic nature of sulfonamide hypersensitivity is evidenced by the presence of drug-specific antibodies in people, although these develop inconsistently in dogs.[62,63] In people and rodents, the major route of detoxification and elimination of sulfonamide antibiotics is acetylation by the N-acetyl-transferase enzymes. Dogs lack the genes for these enzymes and excrete sulfonamide antibiotics largely unchanged, which may explain their susceptibility to the hypersensitivity reaction.[64]

Zonisamide

In 2011, 2 separate case reports described acute onset hepatopathy beginning 10 days and 3 weeks after initiating zonisamide therapy.[65,66] One dog presented with primarily hepatocellular biochemical changes (ALT 16,328 U/L, AST 5908 U/L, and ALP 354 U/L), while the other had a more mixed pattern (ALT 3197 U/L, AST 1275, and ALP 5182 U/L). Both dogs were moderately hyperbilirubinemic (2.3 and 4.3 mg/dL). Both dogs received aggressive treatment with hepatoprotectants and supportive care. One dog made a complete recovery with normalization of liver enzymes after 4 weeks. The other dog was euthanized and massive panlobular hepatic necrosis with marked microvesicular hepatic lipidosis was found on necropsy.

Despite these initial reports, zonisamide-associated DILI is quite rare. In a recent, large, retrospective study, the prevalence of acute, clinical hepatopathy was 0.54%.[67] Additionally, less than 10% of dogs administered zonisamide for at least 3 months had increases in their liver enzymes. Those changes that did develop were relatively mild (ALT 125–200 U/L and ALP 124–746 U/L) and none were associated with clinical

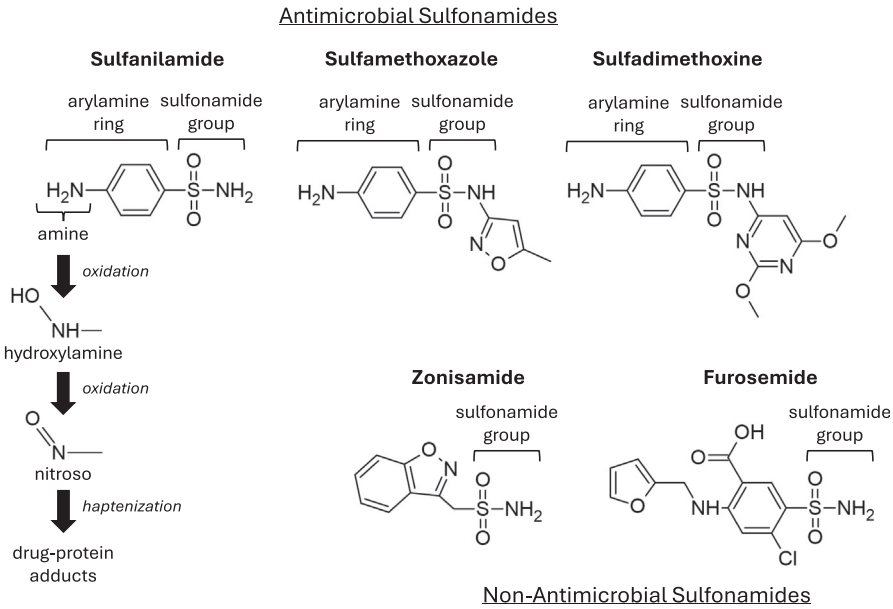

Fig. 2. Biotransformation of sulfonamide antibiotics. All antimicrobial sulfonamides contain an arylamine ring. The amino group on that ring is oxidized to a hydroxylamine by the cytochrome P450 enzymes. Under cellular oxidative conditions, the hydroxylamine group spontaneously degrades into an electrophilic nitroso group, which binds endogenous proteins to form immunogenic drug haptens. Note, nonantimicrobial sulfonamide drugs, like zonisamide, do not contain the arylamine ring and so the pathogenesis of drug-induced liver injury for these drugs must differ from that of sulfonamide antibiotics.

disease. Structurally, zonisamide contains a sulfonamide moiety, and so it has been suggested that zonisamide-associated DILI might share a pathogenesis with sulfonamide antibiotic hypersensitivity.[65] However, like all nonantibiotic sulfonamide drugs, zonisamide lacks the arylamine ring (see **Fig. 1**), which is necessary for haptenization and immune stimulation; so, a different mechanism must underlie zonisamide toxicity.

NUTRACEUTICALS AND HERBAL REMEDIES

Dietary supplements and herbal preparations are commonly used in companion animals, with and without veterinary supervision. Many constituents have known associations with liver injury, and, for others, the hepatotoxic potential has not been evaluated.[68] This is particularly of concern for veterinary nutraceuticals that are minimally regulated in the United States and so are at higher risk for unreported changes in formulation and adulteration.[69] In China, herbal and dietary supplements are the most common cause of DILI in humans and frequencies are increasing in western countries.[1] Thus, veterinarians should be conscious of the toxic potential of any supplement prescribed and be sure to include such products in a thorough drug history.

SUMMARY

DILI is an underrecognized cause of hepatic disease in dogs and cats. Successful identification of cases requires an initial suspicion by the practitioner, a thorough drug and nutraceutical exposure history, and knowledge of the toxic potential and

clinical presentations for common veterinary drugs. There are neither pathognomonic biochemical or histologic changes for DILI nor currently reliable drug-specific diagnostic tests. Therefore, definitive diagnosis is generally made based on resolution of clinical abnormalities following drug withdrawal. For many cases of DILI, prognosis can be good if the offending drug is promptly identified and supportive measures instituted.

CLINICS CARE POINTS

- DILI should be on the differential list for any animal presenting for acute or chronic hepatopathy.
- The most important steps in diagnosing DILI are taking a thorough history and being aware the hepatotoxic potential of commonly used veterinary drugs.
- For intrinsic DILI, therapy with the offending drug can sometimes be reinstituted at a lower dose. For idiosyncratic DILI, the patient should never be exposed to the drug again. When in doubt, chose a different drug.

DISCLOSURE

The author has nothing to disclose.

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REFERENCES

1. Garcia-Cortes M, Robles-Diaz M, Stephens C, et al. Drug induced liver injury: an update. Arch Toxicol 2020;94:3381–407.
2. Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137:947–54.
3. Jaeschke H. Acetaminophen: dose-dependent drug hepatotoxicity and acute liver failure in patients. Dig Dis 2015;33:464–71.
4. Lammert C, Einarsson S, Saha C, et al. Relationship between daily dose of oral medications and idiosyncratic drug-induced liver injury: search for signals. Hepatology 2008;47:2003–9.
5. Vuppalanchi R, Gotur R, Reddy KR, et al. Relationship between characteristics of medications and drug-induced liver disease phenotype and outcome. Clin Gastroenterol Hepatol 2014;12:1550–5.
6. Stephens C, Andrade RJ. Genetic predisposition to drug-induced liver injury. Clin Liver Dis 2020;24:11–23.
7. Reinhart JM, Ekena J, Cioffi AC, et al. A single-nucleotide polymorphism in the canine cytochrome b(5) reductase (CYB5R3) gene is associated with sulfonamide hypersensitivity and is overrepresented in Doberman Pinschers. J Vet Pharmacol Ther 2018;41:402–8.
8. Urban TJ, Shen Y, Stolz A, et al. Limited contribution of common genetic variants to risk for liver injury due to a variety of drugs. Pharmacogenet Genomics 2012; 22:784–95.

9. Cirulli ET, Nicoletti P, Abramson K, et al. A missense variant in PTPN22 is a risk factor for drug-induced liver injury. Gastroenterology 2019;156:1707–16.e2.
10. Gershony LC, Belanger JM, Hytonen MK, et al. Whole genome sequencing reveals multiple linked genetic variants on canine chromosome 12 associated with risk for symmetrical lupoid onychodystrophy (SLO) in the bearded collie. Genes 2021; 12(8):1265.
11. Nakazawa M, Miyamae J, Okano M, et al. Dog leukocyte antigen (DLA) class II genotypes associated with chronic enteropathy in French bulldogs and miniature dachshunds. Vet Immunol Immunopathol 2021;237:110271.
12. Short AD, Boag A, Catchpole B, et al. A candidate gene analysis of canine hypoadrenocorticism in 3 dog breeds. J Hered 2013;104:807–20.
13. Roque JB, O'Leary CA, Kyaw-Tanner M, et al. PTPN22 polymorphisms may indicate a role for this gene in atopic dermatitis in West Highland white terriers. BMC Res Notes 2001;4:571–6.
14. Trepanier LA. Drug-associated liver disease. In: Bonagura JD, Twedt DC, editors. Kirk's current veterinary therapy. 15th edition. St Louis (MO): Elsevier Saunders; 2014. p. 575–9.
15. Chen Y, Guan S, Guan Y, et al. Novel clinical biomarkers for drug-induced liver injury. Drug Metab Dispos 2022;50:671–84.
16. Danan G, Teschke R. RUCAM in drug and herb induced liver injury: the update. Int J Mol Sci 2015;17:14.
17. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981;30:239–45.
18. Worth WS. Azathioprine effect on normal canine liver and kidney function. Toxicol Appl Pharmacol 1968;12:1–6.
19. Wallisch K, Trepanier LA. Incidence, timing, and risk factors of azathioprine hepatotoxicosis in dogs. J Vet Intern Med 2015;29:513–8.
20. Papich MG. Saunders handbook of veterinary drugs. 4th edition. St Louis (MO): Elsevier; 2016.
21. Al Hadithy AF, de Boer NK, Derijks LJ, et al. Thiopurines in inflammatory bowel disease: pharmacogenetics, therapeutic drug monitoring and clinical recommendations. Dig Liver Dis 2005;37:282–97.
22. Kidd LB, Salavaggione OE, Szumlanski CL, et al. Thiopurine methyltransferase activity in red blood cells of dogs. J Vet Intern Med 2008;18:214–8.
23. White SD, Rosychuk RAW, Outerbridge CA, et al. Thiopurine methyltransferase in red blood cells of dogs, cats, and horses. J Vet Intern Med 2008;14:499–502.
24. Legendre AM, Rohrbach BW, Toal RL, et al. Treatment of blastomycosis with itraconazole in 112 dogs. J Vet Intern Med 1996;10:365–71.
25. Rodriguez RJ, Acosta D. N-deacetyl ketoconazole-induced hepatotoxicity in a primary culture system of rat hepatocytes. Toxicology 1997;117:123–31.
26. Somchit N, Wong CW, Zuraini A, et al. Involvement of phenobarbital and SKF 525A in the hepatotoxicity of antifungal drugs itraconazole and fluconazole in rats. Drug Chem Toxicol 2008;29:237–53.
27. Khoza S, Moyo I, Ncube D. Comparative hepatotoxicity of fluconazole, ketoconazole, itraconazole, terbinafine, and griseofulvin in rats. J Toxicol 2017;2017:1–9.
28. Kyriakidis I, Tragiannidis A, Munchen S, et al. Clinical hepatotoxicity associated with antifungal agents. Expert Opin Drug Saf 2016;58:1–17.
29. Mayer UK, Glos K, Schmid M, et al. Adverse effects of ketoconazole in dogs – a retrospective study. Vet Dermatol 2008;19:199–208.

30. Mazepa ASW, Trepanier LA, Foy DS. Retrospective comparison of the efficacy of fluconazole or itraconazole for the treatment of systemic blastomycosis in dogs. J Vet Intern Med 2011;25:440–5.
31. Wilson AG, KuKanich KS, Hanzlicek AS, et al. Clinical signs, treatment, and prognostic factors for dogs with histoplasmosis. J Amer Vet Med Assoc 2018;252:201–9.
32. Reinhart JM, KuKanich KS, Jackson T, et al. Feline histoplasmosis: fluconazole therapy and identification of potential sources of Histoplasma species exposure. J Feline Med Surg 2012;14:841–8.
33. Cresemba (isavuconazonium sulfate) [package insert]. Northbrook, IL: Astrellas Pharma US, Inc; 2022.
34. Noxafil (posaconazole) [package insert]. Whitehouse Station, NJ: Merck & Co., Inc.; 2015.
35. VFEND (voriconazole) [package insert]. New York, NY: Pfizer, Inc; 2010.
36. MacPhail CM, Lappin MR, Meyer DJ, et al. Hepatocellular toxicosis associated with administration of carprofen in 21 dogs. J Am Vet Med Assoc 1998;212:1895–901.
37. Autefage A, Gossellin J. Efficacy and safety of the long-term oral administration of carprofen in the treatment of osteoarthritis in dogs. Rev Med Vet-Toulouse 2007;158:119–27.
38. Mansa S, Palmer E, Grondahl C, et al. Long-term treatment with carprofen of 805 dogs with osteoarthritis. Vet Rec 2007;160:427–30.
39. Bvsc DH, Moreau RE, Overall KL, et al. Acute hepatic necrosis and liver failure associated with benzodiazepine therapy in six cats, 1986–1995. J Vet Emerg Crit Care 2007;6:13–20.
40. Center SA, Elston TH, Rowland PH, et al. Fulminant hepatic failure associated with oral administration of diazepam in 11 cats. J Am Vet Med Assoc 1996;209:618–25.
41. van Beusekom CD, van den Heuvel JJ, Koenderink JB, et al. Feline hepatic biotransformation of diazepam: differences between cats and dogs. Res Vet Sci 2015;103:119–25.
42. Schulz BS, Hupfauer S, Ammer H, et al. Suspected side effects of doxycycline use in dogs - a retrospective study of 386 cases. Vet Rec 2011;169:229.
43. Schulz BS, Zauscher S, Ammer H, et al. Side effects suspected to be related to doxycycline use in cats. Vet Rec 2013;172:184.
44. Kaufman AC, Greene CE. Increased alanine transaminase activity associated with tetracycline administration in a cat. J Am Vet Med Assoc 1993;202:628–30.
45. Hosoya K, Lord LK, Lara-Garcia A, et al. Prevalence of elevated alanine transaminase activity in dogs treated with CCNU (Lomustine). Vet Comp Oncol 2009;7:244–55.
46. Kristal O, Rassnick KM, Gliatto JM, et al. Hepatotoxicity associated with CCNU (Lomustine) chemotherapy in dogs. J Vet Intern Med 2004;18:75–80.
47. Dedeaux AM, Flesner BK, Reinhart JM, et al. Biochemical, functional, and histopathologic characterization of lomustine-induced liver injury in dogs. Am J Vet Res 2020;81:810–20.
48. Skorupski KA, Hammond GM, Irish AM, et al. Prospective randomized clinical trial assessing the efficacy of Denamarin for prevention of CCNU-induced hepatopathy in tumor-bearing dogs. J Vet Intern Med 2011;25:838–45.
49. Musser ML, Quinn HT, Chretin JD. Low apparent risk of CCNU (lomustine)-associated clinical hepatotoxicity in cats. J Fel Med Surg 2012;14:871–5.
50. Peterson ME, Kintzer PP, Hurvitz AI. Methimazole treatment of 262 cats with hyperthyroidism. J Vet Intern Med 1988;2:150–7.

51. Dayrell-Hart B, Steinberg SA, VanWinkle TJ, et al. Hepatotoxicity of phenobarbital in dogs: 18 cases (1985-1989). J Am Vet Med Assoc 1991;199:1060–6.
52. Podell M, Volk HA, Berendt M, et al. 2015 ACVIM small animal consensus statement on seizure management in dogs. J Vet Intern Med 2016;30:477–90.
53. Papich MG. Selection of antibiotics for meticillin-resistant Staphylococcus pseudintermedius: time to revisit some old drugs? Vet Dermatol 2012;23:352–60.e364.
54. Bajwa J, Charach M, Duclos D. Adverse effects of rifampicin in dogs and serum alanine aminotransferase monitoring recommendations based on a retrospective study of 344 dogs. Vet Dermatol 2013;24:570–5.e135–6.
55. De Lucia M, Bardagi M, Fabbri E, et al. Rifampicin treatment of canine pyoderma due to multidrug-resistant meticillin-resistant staphylococci: a retrospective study of 32 cases. Vet Dermatol 2017;28:171-e136.
56. Harbour L, Schick A, Mount R, et al. Rifampicin treatment of canine multidrug-resistant meticillin-resistant staphylococcal pyoderma: a retrospective study of 51 cases. Vet Dermatol 2022;33:384–91.
57. Trepanier LA, Danhof R, Toll J, et al. Clinical findings in 40 dogs with hypersensitivity associated with administration of potentiated sulfonamides. J Vet Intern Med 2003;17:647–52.
58. Twedt DC, Diehl KJ, Lappin MR, et al. Association of hepatic necrosis with trimethoprim sulfonamide administration in 4 dogs. J Vet Intern Med 1997;11:20–3.
59. Cribb AE, Spielberg SP. An in vitro investigation of predisposition to sulphonamide idiosyncratic toxicity in dogs. Vet Res Comm Commun 1990;14:241–52.
60. Giger U, Werner LL, Millichamp NJ, et al. Sulfadiazine-induced allergy in six Doberman pinschers. J Amer Vet Med Assoc 1985;186:479–84.
61. Trepanier LA. Idiosyncratic toxicity associated with potentiated sulfonamides in the dog. J Vet Pharmacol Therapeut 2004;27:129–38.
62. Daftarian MP, Filion LG, Cameron W, et al. Immune response to sulfamethoxazole in patients with AIDS. Clin Diagn Lab Immunol 1995;2:199–204.
63. Lavergne SN, Danhof RS, Volkman EM, et al. Association of drug-serum protein adducts and anti-drug antibodies in dogs with sulphonamide hypersensitivity: a naturally occurring model of idiosyncratic drug toxicity. Clin Exp Allergy 2006;36:907–15.
64. Trepanier LA, Ray K, Winand NJ, et al. Cytosolic arylamine N-acetyltransferase (NAT) deficiency in the dog and other canids due to an absence of NAT genes. Biochem Pharmacol 1997;54:73–80.
65. Miller ML, Center SA, Randolph JF, et al. Apparent acute idiosyncratic hepatic necrosis associated with zonisamide administration in a dog. J Vet Intern Med 2011;25:1156–60.
66. Schwartz M, Muñana KR, Olby NJ. Possible drug-induced hepatopathy in a dog receiving zonisamide monotherapy for treatment of cryptogenic epilepsy. J Vet Med Sci 2011;73:1505–8.
67. Smith TK, Cameron S, Trepanier LA. Incidence of hepatopathies in dogs administered zonisamide orally: a retrospective study of 384 cases. J Vet Intern Med 2022;36:576–9.
68. Nunes D, Monteiro CSJ, Dos Santos JL. Herb-induced liver injury-a challenging diagnosis. Healthcare (Basel) 2022;10:278.
69. Finno CJ. Veterinary pet supplements and nutraceuticals. Nutr Today 2020;55:97–101.