NOTE *Internal Medicine*

Possible Drug-Induced Hepatopathy in a Dog Receiving Zonisamide Monotherapy for Treatment of Cryptogenic Epilepsy

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ABSTRACT. A 9-year old female spayed Rottweiler was diagnosed with cryptogenic epilepsy and started on zonisamide monotherapy (8.3 mg/kg, PO, q 12 hr). Three weeks after the 1st dose of zonisamide the dog presented for vomiting, inappetence and icterus. Serum biochemistry showed marked elevation of liver enzymes, consistent with hepatocellular damage and cholestasis. No underlying cause for liver disease was identified and a drug-induced hepatopathy was suspected. Zonisamide was discontinued and replaced by potassium bromide. Supportive therapy consisted of intravenous fluids, antiemetics, antibiotics and hepatoprotectants. The dog made a complete recovery and serial serum biochemical examinations showed complete normalisation of liver parameters 8 weeks after discontinuation of zonisamide. Based on a human Drug-induced Liver Injury Diagnostic Scale, the likelihood for zonisamide-induced hepatopathy was classified as "possible". Veterinary practitioners and owners should be educated about the potential for an idiosyncratic drug reaction to zonisamide. If signs of hepatotoxicity are recognised early and zonisamide is discontinued, complete recovery is possible.
KEY WORDS: antiepileptic drug, canine, idiosyncratic drug reaction, liver, seizure.

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The sulfonamide derivate zonisamide (ZNS) is a new generation antiepileptic drug (AED) that was introduced into the Japanese market in 1989 and received licensure in the US and Europe in 2000 and 2005, respectively. While the US and European approval is limited to use as an add-on AED for humans with partial seizures, the Japanese license includes utilisation as mono- and adjunctive therapy for partial and generalised seizures [5].

With blockage of voltage-sensitive sodium channels and T-type calcium channels, ZNS possesses a unique mode of action among the currently available AEDs. There is evidence that ZNS also exhibits direct effects on synthesis, release and degradation of the neurotransmitters glutamate, gamma aminobutyric acid (GABA), dopamine, serotonin and acetylcholine, thereby promoting synaptic inhibition. Furthermore, ZNS has been ascribed neuroprotective effects. To date it remains uncertain as to whether the direct effects on neurotransmitters and the neuroprotective properties play a role in the clinical use as an AED [1].

In contrast to many of the newly developed anticonvulsants, ZNS has a sufficiently long serum elimination half-life in dogs, to allow maintenance of therapeutic blood levels by means of twice daily dosing [2, 9]. This feature in particular has raised interest in using ZNS for treatment of canine epilepsy, and assessment of its use as add-on medication in cases of refractory epilepsy provided promising results with good rates of responders [3, 11].

Throughout the reports on the use of ZNS in dogs, the drug appears to have a favourable adverse effect profile. Side effects, including ataxia, lethargy and vomiting, were mild and transient and never warranted discontinuation [3, 11]. A study on the chronic toxicity of ZNS in dogs, using over 7 times the recommended dose for a duration of 52 weeks, revealed mild effects on the liver only that were not associated with any significant clinical signs [12].

The purpose of this report is to describe the findings in a dog with severe hepatopathy that developed following initiation of ZNS monotherapy and resolved after discontinuation of ZNS with supportive care.

A 9-year old female spayed Rottweiler (36 kg) presented to the Neurology Service of the Veterinary Teaching Hospital (VTH), North Carolina State University for investigation and treatment of generalised cluster seizures that began 36 hr prior to presentation.

The patient had no significant clinical history and was up-to-date with standard vaccinations and parasite treatment and prevention. Current medication consisted of carprofen (2.1 mg/kg, PO, q 24 hr (Rimadyl; Pfizer)) and a glucosamine-containing joint supplement (1 tablet q 24hr (Glyco-Flex II; Vetri-Science Laboratories)) for treatment of bilateral coxofemoral joint osteoarthritis, phenylpropanolamine (1.4 mg/kg, PO, q 24 hr (Proin 50 Chewable Tablets; PRN Pharmacal)) for treatment of urinary incontinence and famotidine (0.6 mg/kg, PO, q 12 hr (Famotidine Tablets USP; Ivax Pharmaceuticals Ireland)) for treatment of an episode of vomiting. All medications were started 4–6 months before presentation to the VTH. The owner had not noticed any changes preceding the onset of seizures and intoxication was deemed highly unlikely. The first seizure was generalised tonic-clonic and lasted approximately 2 min. It was followed by post-ictal signs of aggressiveness that persisted for about 3 ½ hr. The dog was taken to the primary veterinarian where a general physical and neurological examination were unremarkable, except for discomfort upon

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manipulation of both coxofemoral joints. Hematological and serum biochemical evaluation (including total T4) as well as urinalysis did not reveal any abnormalities except for mildly increased cholesterol (396 mg/d*l*; reference range 92–324) and a low urine specific gravity (1.012). Liver enzymes and total bilirubin were as follows: aspartate aminotransferase (AST) 42 U/*l* (15–66), alanine aminotransferase (ALT) 25 U/*l* (12–118), alkaline phosphatase (ALKP) 58 U/*l* (5–131), γ-glutamyltransferase (γ-GT) 5 U/*l* (1–12), total bilirubin 0.1 mg/d*l* (0.1–0.3). Over the following 24 hr the dog experienced a total of 4 more isolated seizures, which prompted referral to the VTH.

The general physical examination was unremarkable, except for signs of coxofemoral osteoarthritis. A fundic examination did not show any abnormalities, and 3 consecutive non-invasive blood pressure measurements were within normal limits. The neurological examination revealed slight paraparesis and proprioceptive hindlimb ataxia with mild signs of discomfort on palpation of the cranial lumbar spine and unremarkable spinal reflexes. No cranial nerve deficits were present. The neurological lesion localization was forebrain with possible additional T3 – L3 myelopathy. Main categories of differential diagnoses included neoplastic, vascular, cryptogenic, metabolic/toxic and infectious/inflammatory for the forebrain lesion, and degenerative and neoplastic for a spinal cord lesion, which was considered an incidental finding and not further investigated. At the end of the examination, the dog experienced a generalised seizure and a single dose of diazepam (1 mg/kg (Diazepam Injection, USP; Hospira)) was administered rectally and intravenous access was established. The blood glucose concentration was 101 mg/d*l* (70–131). To help prevent further seizures, a single dose of phenobarbital was given (4 mg/kg, IV (Phenobarbital Sodium Injection, USP; Baxter)). The same day, thoracic and spinal radiographs were performed under sedation (midazolam 0.5 mg/kg, IV (Midazolam HCl Injection; Baxter) + butorphanol 0.1 mg/kg, IV (Torbugesic; Pfizer)) and did not reveal any abnormalities except for degenerative changes to multiple joints. A bile acid tolerance test showed pre-prandial bile acids of 7.8 μmol/*l* (0–20) and post-prandial bile acids of 38.2 μmol/*l* (0–30). However, the serum from the post-prandial sample was not separated from the blood cells overnight and the result was therefore considered unreliable [10]. The dog was started on ZNS (9.7 mg/kg, PO, q 12 hr total of 4 doses over 2 days (Zonisamide Capsules; Sun Pharmaceutical + Zonisamide Capsules; Wockhardt)) and magnetic resonance imaging of the brain and cisternal cerebrospinal fluid analysis were performed the following day, both of which were unremarkable. A diagnosis of cryptogenic epilepsy was established and the dog, which did not have any more seizures while hospitalised, was discharged on ZNS therapy (8.3 mg/kg, PO, q 12 hr (Zonisamide Capsules; Sun Pharmaceutical)). It was recommended to repeat measurement of post-prandial bile acids and to perform an abdominal ultrasound to further rule out extracranial causes for seizures. At this point, the owner declined both of these tests.

Three weeks after the first dose of ZNS, the dog started vomiting and became inappetant and icteric. No abnormalities were observed prior to this change and the dog had not had any seizure activity since discharge from the VTH. Again the owner felt that toxin exposure was highly unlikely. A serum biochemical examination at the primary veterinarian showed markedly elevated liver enzymes (AST 1275 U/*l*; ALT 3197 U/*l*; ALKP 5182 U/*l*; γ-GT 23 U/*l*) with an increase in total bilirubin (4.3 mg/d*l*) and cholesterol (700 mg/d*l*). The remaining values were within reference ranges. The dog re-presented to the VTH where the general physical and neurological examination were unchanged from the previous visit aside from a marked icterus. A coagulation panel showed values within normal limits (prothrombin time 8.7 s (6.8–10.7), activated partial thromboplastin time 11.6 s (7.5–13.8)). An abdominal ultrasound visualised unremarkable liver parenchyma with moderately enlarged hepatic lymph nodes. The wall of the neck of the gall bladder appeared mildly thickened. Based on these findings a focal cholecystitis was considered possible. Fine needle aspirates of the liver were cytologically unremarkable. No cause for the acute onset of liver disease could be established and an adverse drug reaction to ZNS was considered possible. The dog was hospitalised until the following day, and non-specific supportive therapy consisting of intravenous fluid therapy (0.45% NaCl + 1.8 mEq KCl at 4 m*l*/kg/h while hospitalised), an antiemetic (maropitant, 1 mg/kg, SC, q 24 hr while hospitalised and 2.2 mg/kg, PO, q 24 hr for 3 days (Cerenia; Pfizer)), antibiotics (amoxicillin/clavulanic acid, 13.9 mg/kg, PO, q 12 hr for 14 days (Clavamox; Pfizer) and ciprofloxacin, 10.4 mg/kg, PO, q 12 hr for 14 days (Ciprofloxacin Tablets USP; Unique Pharmaceutical Laboratories)), and hepatoprotectants (ursodiol, 5.6 mg/kg, PO, q 12 hr for 14 days (Ursodiol USP; PCCA), and S-Adenosyl-Methionine, SAMe, 18.8 mg/kg, PO, q 24 hr for 14 days (Denosyl; Nutramax Laboratories)) was initiated. ZNS was discontinued and antiepileptic treatment with potassium bromide (Potassium Bromide Purified Granular; PCCA) was started. A 2-day loading phase (8 × 55 mg/kg, PO, q 6 hr) was followed by maintenance dosing (28 mg/kg, PO, q 24 hr). In the event of a seizure the dog was to be given levetiracetam (28 mg/kg, PO, q 8 hr for 2 days (Levetiracetam Tablets; Glenmark Generics)) to prevent cluster seizures.

Following discharge, the dog made a complete recovery and serial serum biochemical examinations at the primary veterinarian showed a gradual normalisation of liver values. Four weeks after discharge, the dog was clinically unremarkable and liver values were as follows: AST 34 U/*l*, ALT 127 U/*l*, ALKP 707 U/*l*, γ-GT 8 U/*l*, total bilirubin 0.2 mg/d*l*. Cholesterol concentration remained mildly elevated (345 mg/d*l*). At 8 weeks, another re-check showed complete normalisation of the liver parameters tested (ALT 30 U/*l*, ALKP 120 U/*l*, total bilirubin 0.1 mg/d*l*).

Main categories of adverse drug effects are type A (pharmacology-related) and type B (idiosyncratic). While type A effects are dose dependant, occur predictably and are caused

by a known pharmacological property of the agent, idiosyncratic reactions cannot be explained on the basis of the known mechanisms of action of the drug and occur mostly unpredictably in susceptible individuals only, irrespective of dosage [14]. Idiosyncratic reactions are usually caused by either immune-mediated hypersensitivity reactions or by cytotoxic effects of the drug or one of its metabolites. Due to the liver's central role in drug metabolism it is one of the major sites where idiosyncratic drug reactions manifest. Individual differences in rate of formation and detoxification of reactive metabolites may explain why only certain patients develop idiosyncratic reactions [14]. These unpredictable adverse reactions occur rarely and therefore frequently remain undetected during clinical trials until approval and marketing of drugs. Once a large number of patients is exposed to the new agents these rare adverse effects may emerge [7].

Establishment of drug-induced liver injury is problematic and proof of causality usually requires re-challenge, which clearly is dangerous and should be avoided. Therefore clinical scales have been developed for humans that determine likelihood for a causative relationship. Maria and Victorino have proposed and validated a scale based on the following criteria: (a) temporal relationship of initiation/discontinuation of drug therapy and onset/resolution of signs of liver injury, (b) exclusion of alternative causes, (c) occurrence of extra-hepatic signs of adverse drug reaction, (d) results of drug re-exposure, and (e) whether similar adverse effects have been reported previously (Table 1) [6]. When applied to the case reported here, high scores were obtained in the category for temporal relationship of occurrence and resolution of clinical signs (9/9). Alternative causes for hepatocellular damage with signs of cholestasis were investigated by means of abdominal ultrasound and fine-needle aspirates of

Table 1. Components of the Drug-Induced Liver Injury Diagnostic Scale (Maria and Victorino 1997) relevant to the case described

CRITERIA	SCORE (points)
1. Temporal Relationship Between Drug Intake and Onset of Clinical Picture	
A. Time from drug intake until onset of clinical or laboratory manifestation	
a. 4 days to 8 weeks (or less than 4 days in cases of re-exposure)	+3
b. Less than 4 days or more than 8 weeks	+1
B. Time from withdrawal of drug until onset of manifestation	
a. 0 to 7 days	+3
b. 8 to 15 days	0
c. More than 15 days	–3
C. Time from withdrawal of drug until normalization of laboratory values	
a. Less than 6 months (cholestatic or mixed) or 2 months (hepatocellular)	+3
b. More than 6 months (cholestatic or mixed) or 2 months (hepatocellular)	0
2. Exclusion of Alternative Causes	
a. Complete exclusion	+3
b. Partial exclusion	0
c. Possible alternative cause detected	–1
d. Probable alternative cause detected	–3
3. Extrahepatic Manifestation	
Rash, fever, arthralgia, eosinophilia (>6%), cytopenia	
a. 4 or more	+3
b. 2 or 3	+2
c. 1	+1
d. None	0
4. Intentional or Accidental Re-exposure to Drug	
a. Positive re-challenge test	+3
b. Negative or absent re-challenge test	0
5. Previous Report of Drug-induced Liver Injury Associated with the Drug	
a. Yes	+2
b. No (drug marketed for ≤ 5 years)	0
c. No (drug marketed for > 5 years)	–3

TOTAL SCORE	DEGREE OF PROBABILITY OF ADVERSE DRUG REACTION
> 17	Definite
14–17	Probable
10–13	Possible
6–9	Unlikely
< 6	Excluded

the liver. A neoplastic cause seems unlikely given the ultrasonographical appearance of the liver and the favourable response to non-specific therapy. An infectious cholangiohepatitis and cholecystitis causing the dog's clinical signs cannot be ruled out although this disorder is not encountered frequently in dogs [8]. In fact, this possibility prompted the decision to initiate antimicrobial therapy. Liver biopsies and bile cultures would have helped to render an infectious cause less likely. Exposure to another hepatotoxin cannot entirely be excluded, although in-depth questioning of the owner did not suggest any likely agents [4]. Alternative causes for hepatopathy have thus been partially excluded, which scores 0/3 points in the Drug-induced Liver Injury Diagnostic Scale [6]. The absence of proposed extrahepatic signs (rash, pyrexia, arthralgia, eosinophilia, cytopenia) scores 0/3 points, as does omission of re-challenge. No reports of hepatotoxicity induced by ZNS exist for the dog, however, liver toxicity has been reported as a serious idiosyncratic reaction in humans [14] and in fact the label of the ZNS medication Zonegran (2009 version) includes a warning of potentially fatal fulminant hepatic necrosis. Presence of reports of hepatotoxicity associated with ZNS earns 2/2 points in the scoring system. With a total score of 11 out of 20 possible points, the probability that the case reported here represents a true drug-induced liver injury is classified as "possible" (Table 1) [6].

Zonisamide blood concentration was not determined for the presented case and, currently, ZNS blood concentrations are not routinely measured among veterinary neurologists in the United States. Nevertheless, this may become very useful once more canine-specific data on therapeutic ZNS blood levels are available. Based on the lack of signs of toxicity with chronic administration of much higher doses than chosen for this patient [12], it appears unlikely that a dose-dependant toxicity has occurred in this dog. The dog was administered general supportive therapy for liver disease. The cytoprotective agents SAMe and ursodiol were used for their positive effects in necrotizing and inflammatory hepatopathies in dogs. Main beneficial mechanisms of action, among others, are indirect antioxidant properties of SAMe and choleretic qualities of ursodiol [13]. The authors acknowledge that it has not been proven that the dog's liver injury was caused by treatment with ZNS and only re-challenge could have provided this. In addition, the purpose of this report is not to discourage the use of ZNS in dogs. In fact, ZNS was selected as a first line AED in this dog because it is well-tolerated, shows fewer side effects than the conventional AEDs phenobarbital and potassium bromide, and appears efficacious. Therefore, the intention of reporting this case is to alert the veterinary community of the potential for an idiosyncratic drug reaction to ZNS. Educating owners and veterinary practitioners will allow close monitoring and early recognition of signs compatible with hepatotoxicity. The described case demonstrates that recovery is possible if drug administration is discontinued immediately and supportive therapy initiated.

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