The Journal of Veterinary Medical Science

NOTE

Pathology

Hepatocellular necrosis with prominent regenerative reactions in a zonisamide administrated dog

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J. Vet. Med. Sci.
82(6): 704–706, 2020
doi: 10.1292/jvms.20-0045

Received: 26 January 2020
Accepted: 26 March 2020
Advanced Epub:
3 April 2020

ABSTRACT. A 16 years old neutered male Miniature Dachshund with 1-year history of repetitive administration of zonisamide for treatment of epileptic seizure was presented for vomiting, anorexia and diarrhea. Serum biochemistry showed a markedly elevated ALP level. The dog died 6 days after the presentation and a necropsy was performed. Histopathologically, random, focal to extensive necrosis, formation of regenerative hepatocellular nodules surrounded by fibrous septa and proliferation of bile ducts were seen in the liver. From these findings, the hepatic lesion was diagnosed as hepatocellular necrosis with prominent regenerative reactions due to the chronic persistent liver injury. Hepatic lesions were considered to be induced by zonisamide, based on the history of continuous administration, and clinical and histopathological findings.

KEY WORDS: dog, hepatocellular necrosis, liver, zonisamide

Zonisamide is sulfonamide-based new-generation antiepileptic drug utilized for treatment of seizures associated with epilepsy. It was approved for human use in 1998, then launched in 2014 for treatment of dog seizures [2]. The drug tends to have mild side effects [7]. In a chronic toxicity study in dogs, even over dose of zonisamide showed relatively good safety range with only mild effects on the liver [9]. Here we present an unusual case of hepatocellular necrosis with prominent regenerative reactions, due to the chronic liver injury in a zonisamide administrated dog.

A 15-year-old neutered male Miniature Dachshund developed epileptic seizure and was referred to the private veterinary hospital. The dog started on zonisamide treatment (3.0 mg/kg, PO, BID), and the dose had increased (8.3 mg/kg, PO, BID) depending on the frequency of seizures. The dose of zonisamide was within the specified range. Twenty days after the first treatment by zonisamide, mild liver enlargement was observed by X-ray, with an increase level of alkaline phosphatase (ALP) (653 U/*l*; reference range, 49–298 U/*l*) (Supplementary Table 1). Four months later, an abdominal ultrasonography revealed a high echoic liver parenchyma with diffuse low echoic nodules. The liver continued to be enlarging and the number of nodules had gradually increased. Approximately 1 year after the first treatment by zonisamide, a serum biochemical examination showed a markedly elevated level of ALP (3,854 U/*l*), aspartate aminotransferase (AST) (101 U/*l*; reference range, 18–65 U/*l*), alanine aminotransferase (ALT) (1,029 U/*l*; reference range, 20–99 U/*l*), C-reactive protein (CRP) (3 mg/d*l*; reference range, 0–1 mg/d*l*), and the dog developed vomiting, diarrhea and anorexia. The dog had continued zonisamide administration to control epilepsy. The condition of the dog worsened and was admitted to the Osaka Prefecture University Veterinary Medical Center (OPU-VMC). Five days later, the dog presented dyspnea and chest X-ray revealed an excessive impermeability of the lung field. Liver enzymes and white blood cell (WBC), CRP were altered as follows: AST (122 U/*l*), ALT (317 U/*l*), ALP (4,559 U/*l*), WBC (47,200 /μ*l*; reference range, 5,200–13,900 /μ*l*) and CRP (13.32 mg/d*l*). Other parameters were within reference range. Antinuclear antibody test and rheumatoid factor were negative. The dog died 6 days after admission to the OPU-VMC.

At necropsy, the liver was enlarged and red or orange-red multiple nodules of 1–2.5 cm in diameter were seen within the whole liver (Fig. 1). Edema and congestion were noted in the lungs. Tissue specimens were collected from the liver, spleen, kidneys, heart, lungs, stomach, small and large intestines, pancreas and adrenal glands. They were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Five μm sections were stained with hematoxylin and eosin (HE).

Formalin-fixed liver sections were also stained with Sirus Red staining and cytokeratin (CK) 19 immunohistochemistry using

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(Supplementary material: refer to PMC http://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

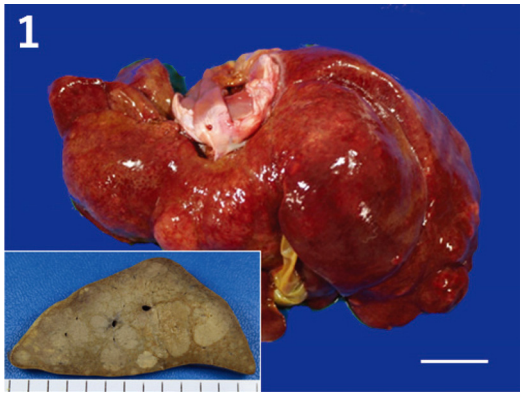

Fig. 1. Gross appearance of the dog's liver. The liver is enlarged and red or orange-red 10 multiple nodules of 1–2.5 cm in diameter are seen within the whole liver. Bar, 2 cm. Inset: cut surface of fixed liver shows grayish nodular lesions.

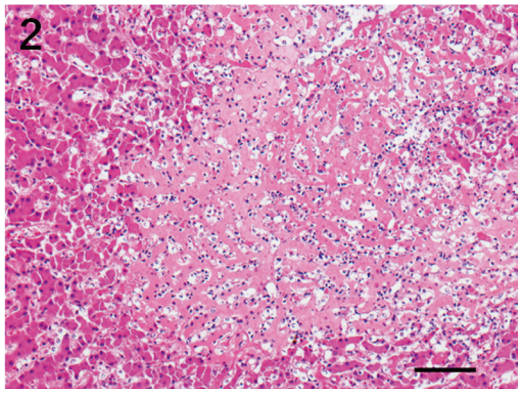

Fig. 2. Histopathological feature of the liver. Random focal to extensive necrosis is seen. Hematoxylin and eosin. Bar, 100 μm.

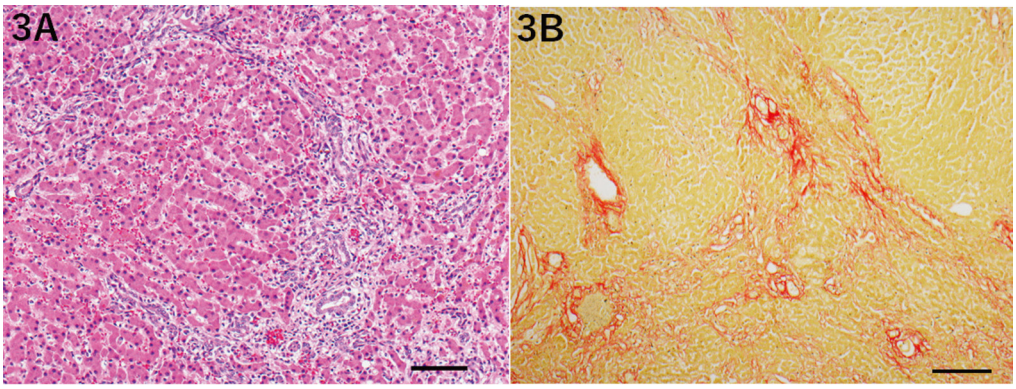

Fig. 3. (A) Histopathological feature of the nodular regenerative lesions. The nodules are surrounded by fibrous septa and proliferating bile ducts. Hematoxylin and eosin. Bar, 100 μm. (B) Extensive fibrous septa are stained red with Sirus Red staining. Bar, 200 μm

anti CK19 mouse monoclonal antibody (Leica Biosystmes, B170, 1:100, Nussloch, Germany) and Simple stain Max-PO (Nichirei, Tokyo, Japan).

Histopathological examination of the liver revealed randomly distributed focal to extensive hepatocellular necrosis with infiltration of neutrophils (Fig. 2) and a few bacterial colonies in the necrotic lesions. Nodules consisting of regenerative hepatocytes surrounded by fibrous septa with proliferating bile ducts were seen (Fig. 3A). The fibrous septa were stained red with Sirus Red staining (Fig. 3B). The architecture of hepatic lobules was unclear. Proliferating bile ducts stained positively with CK19 consisted of non-atypical biliary epithelia (Fig. 4A and 4B). Hepatocytes around the proliferating bile ducts were atrophic with many lipofuscin pigments. Hemosiderin-laden macrophages and Kupffer cells were scattered. The lung had severe alveolar edema and infiltration of neutrophils and fibrin, formation of hyaline membrane were seen in the alveoli.

From these findings, the hepatic lesion was diagnosed as hepatocellular necrosis with prominent regenerative reactions due to the chronic persistent liver injury. The present case was characterized by random and focal necrosis of hepatocytes with proliferation of non-atypical hepatocytes and bile ducts. Proliferation of hepatocytes and bile ducts was considered as compensated reaction to persistent and repetitive hepatocellular damage. Bacterial colonies were distributed in very restricted necrotic areas. Thus, infection may be considered as a secondary lesion and occurred just before the death.

In this case, continuous administration of zonisamide is considered as a main cause of the liver injury. In the limited cases of zonisamide-induced acute liver injury in dogs, anorexia, vomiting [3, 4] and a marked increase in liver enzyme levels (ALT, AST and ALP) [3] were reported as clinical features. As histopathological features, massive panlobular hepatic necrosis, moderate oval cell hyperplasia, and congestion attributed to lobular collapse and stagnation of sinusoidal blood flow [3] were also reported. As mentioned above, a chronic toxicity study of zonisamide in dogs, administering continuously over seven times of the recommended dose, reported a reduced food consumption and an increase in ALP level as clinical signs, with mild hepatocellular cytoplasmic vacuolation as a histological feature [9]. Considering the administration of zonisamide and chronic progressive hepatic lesions,

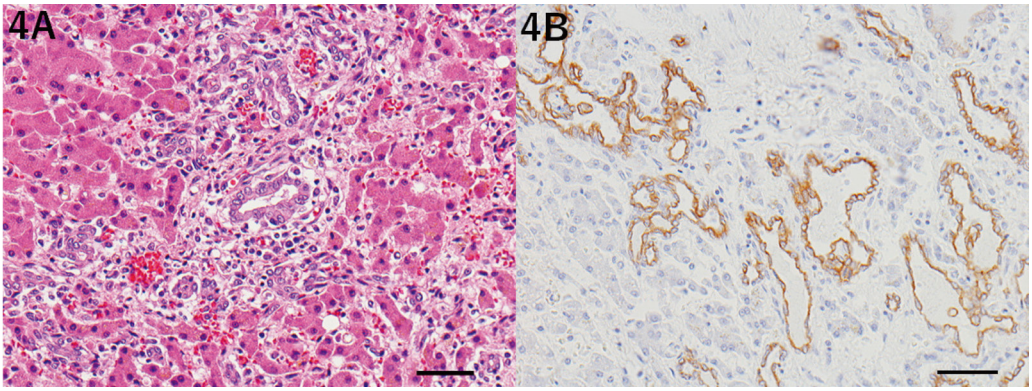

Fig. 4. (A) Histopathological feature of proliferating bile ducts. They consist of non-atypical biliary epithelia. Hematoxylin and eosin. Bar, 50 μm. (B) Proliferating bile ducts are stained positively with cytokeratin 19 immunohistochemistry. Bar, 50 μm.

zonisamide is considered as a main cause of the liver injury in the present case. Although zonisamide is widely used antiepileptic drug in dogs, there are few reports about liver toxicity. Therefore, we suspected some idiosyncratic predisposition may also be involved in the occurrence zonisamide-induced hepatotoxicity.

In addition, zonisamide is commonly used in humans, and there are some reports on adverse effects of zonisamide. However, pathogenesis for zonisamide-induced hepatotoxicity still remains controversial. In human cases, elevated liver enzyme levels such as ALT, AST [1, 5, 8], and ALP [5, 8] and jaundice [1, 8], focal hepatocellular necrosis [1], cholestasis and vanishing bile duct syndrome [8] were reported. In one case of them [1], R-value (a criterion for pathogenesis of drug-induced liver injury) was checked, and it was compatible with hepatocellular damage. On the other hand, in another human case of zonisamide-induced liver injury [8], the liver injury was thought to be classified as drug-induced cholestatic liver disease, characterized by predominant elevations of ALP level secondary to the administration of a hepatotoxic agent [6]. The present case also showed a prominently elevated ALP, and less R-value (Supplementary Table 1), mimicking cholestatic drug-induced liver injury in humans.

In conclusion, this is the report of a canine case diagnosed as hepatocellular necrosis with prominent regenerative reactions due to the chronic liver injury probably induced by zonisamide, based on the history of continuous administration of zonisamide, clinical and histopathological findings. To the best of the authors knowledge, zonisamide-induced chronic liver injury with bile duct proliferation has not been reported in the dogs. Additionally, it is worthy to note that the dog was treated within the specified range. Therefore, it is important to consider that unusual and unexpected excess liver damage may occur in zonisamide-treated dogs.

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