

<Case Report>

## Cerebellar encephalopathy from diminazene aceturate (beneril) toxicity in a dog

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**Abstract :** A 2-year old castrated male Alaskan malamute was referred with primary complaints of marked anemia, hemeglobinuria and depression. Laboratory tests revealed canine babesiosis with severe anemia. The dog was treated by blood transfusion and beneril (diminazene aceturate, 3.5 mg/kg IM). Two days after Beneril injection, the dog suddenly showed ataxia progressing to paresis. MRI revealed irregularly diffused lesions in the cerebellum. The case was tentatively diagnosed as cerebellar encephalopathy caused by diminazene toxicity. The dog successfully recovered following steroid therapy.

**Keywords:** beneril, cerebellar ataxia, cerebellar encephalopathy, diminazene aceturate, dog

Canine babesiosis is a tick-transmitted disease caused by the intra-erythrocytic protozoan parasites *Babesia (B.) canis* and *B. gibsoni* [6]. Canine babesiosis occurs worldwide including Korea [10]. Hemolytic anemia and hypotensive shock are common clinical consequences of infection [13]. The common medications against canine babesiosis are diminazene aceturate (Beneril), phenamidine isethionate, and imidocarb dipropionate, although the efficacy of these drugs is different from the babesia species and strains [7]. The diminazene aceturate is most popular drug for treating canine babesiosis in Korea.

Diminazene aceturate is anti-protozoan agent, which is widely used to treat babesiosis [10] and trypanosomiasis [9, 12]. It may interfere aerobic glycolysis and DNA synthesis of protozoa, although the exact mechanism of action has yet been clearly understood. Although it may not completely eradicate the target protozoa, it can suppress recurrence of clinical signs. Parenteral administration of diminazene often caused acute clinical signs including vomiting and diarrhea, although the occurrence of side effect is less than other anti-protozoan agents [12, 14]. Because diminazene has a low therapeutic index, it often caused fatal nervous complication after 24~48 h of overdose. Clinical signs associated with diminazene toxicity are depression or stupor, continuous vocalization, ataxia, opisthotonos, extensor rigidity, nystagmus and seizures [2]. Although higher dose, repeated doses and intravenous administration of diminazene toxicity can induce toxicity more easily, the toxicity can be occurred by

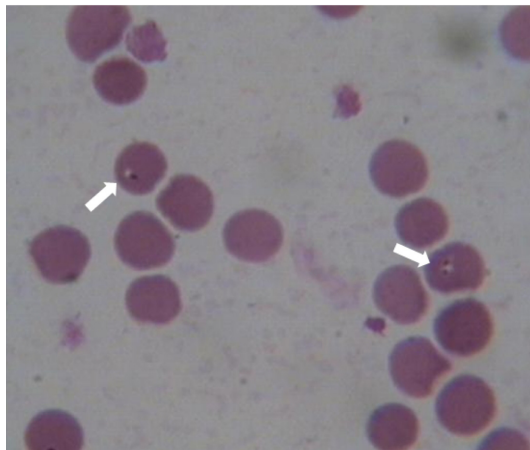
lower dose, single dose and intramuscular administration in dogs. This case study is also described diminazene toxicity induced by single recommended dose with intramuscular administration and confirmed by magnetic resonance imaging (MRI) studies in a dog.

A 2-year old castrated male Alaskan malamute dog (weighing 25 kg) was referred to Veterinary Teaching Hospital of Kangwon National University, with primary complaints of depression, anorexia, hemoglobinuria and anemia. Physical examination showed tachycardia (130 beats per min) and hypotension (100 mmHg, systolic blood pressure measured by Doppler method). Complete blood cell counts revealed regenerative hypochromic anemia (red cell counts  $3.2 \times 10^{12}/\mu\text{L}$  reference  $5.5\text{--}8.9 \times 10^{12}/\mu\text{L}$ ; hematocrit 22.7%, reference 37~55%; hemoglobin: 10.5 g/dL, reference: 12.8~19.6 g/dL) with increased number of reticulocytes. Blood smears found red cells infected with *Babesia* spp., and regenerative changes including polychromasia and anisocytosis (Fig. 1). Urinaysis found 2+ bilirubinuria and hemoglobiuria. Serum biochemistry found hyperglycemia (glucose 225 mg/dL, reference: 77~125 mg/dL), mild hyponatremia (sodium 137 mmol/L, reference: 144~160 mmol/L), mild hyperproteinemia (total protein 8.6 g/dL, reference 5.2~8.2 g/dL) and mild elevation of hepatic enzymes (alanine transaminase 225 U/L, reference 20~80 U/L; aspartate transaminase 400 U/L, reference 15~60 U/L). Due to suspicion of babesiosis, the blood sample was submitted to polymerase chain reaction (PCR) test for confirmation of babesiosis. The PCR test con-

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**Fig 1.** Babesia in red blood cell (RBC) of this case. Round and oval form (white arrows) was showed in RBC that was stained by diff-quick.

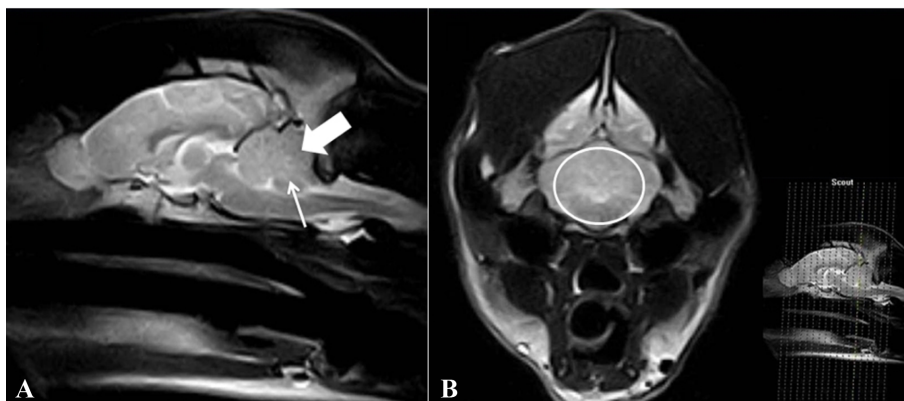
firmed *B. gibsoni* infection for this dog.

The dog was immediately treated with whole blood transfusion (target hematocrit to 37%). Next day, to eradicate babesiosis, diminazene aceturate (Beneril 3.5 mg/kg) was injected intramuscularly. The dog was released. Two days after diminazene treatment, the dog then was re-visited with primary complaints of ataxia, nystagmus and occasional seizures. The dog was immediately treated with diazepam (1 mg/kg, IV) and normal saline infusion. The clinical signs were worsen with time. Also the dog showed more severe neurological signs including paresis. Phenobarbital (6 mg/kg, IV) and methylprednisolone sodium succinate (30 mg/kg, IV) were administered to control neurological signs and possible immune-mediated side-effects. The neurological signs were lessened after this therapy. The doses of phenobarbital and methylprednisolone sodium succinate were tapered with time to 2 mg/kg and 10 mg/kg, respectively. No particular abnormalities have been observed in routine laboratory tests, except increased number of red blood cell counts ( $4.8 \times 10^{12}/\mu\text{L}$ ) by earlier transfusion therapy. Although the clinical

signs were markedly improved with phenobarbital and steroid therapy, the ataxia persisted in this dog. Therefore, we did brain MRI study on this dog. The brain MRI study found diffuse lesion with irregular marginal changes at cerebellum on the sagittal T2 flair image (Fig. 2A) and lesion of high signal at cerebellum on transverse T2 flair image (Fig. 2B). Concurrent cerebrospinal fluid (CSF) analysis found high total protein (192 mg/dL, reference: 0~30 mg/dL) and high red cell counts (42 cells/ $\mu\text{L}$ , reference: 0~30 cells/ $\mu\text{L}$ ). Based on the brain MRI and CSF analysis, we confirmed cerebellar encephalopathy induced by diminazene toxicity in this case, although several brain diseases (e.g. granulomatous meningoencephalitis, degenerative myeloencephalopathy) could cause similar clinical consequences in dogs. Because medical history and clinical findings with and MRI study were strongly suggested berenil toxicity, we did not perform further tests for ruling out other tick-borne disease. Because the dog was responsive to phenobarbital and steroid therapy, we kept medicating phenobarbital (tapering to 1 mg/kg PO, BID) and prednisolone (tapering to 1 mg/kg PO, BID) until the clinical condition was stable. After 4 weeks of therapy, we discontinued all medication. The dog is clinically normal except very mildly ataxic.

Babesia infections in dogs are caused by larger piroplasms collectively described as *B. canis* (3~5  $\mu\text{m}$ ) and smaller piroplasma that are often described as *B. gibsoni* (0.5~2.5  $\mu\text{m}$ ) [2, 3]. Infection with *B. gibsoni* occurs more commonly in American Staffordshire and American pit bull terriers in the USA, probably because of high risk of direct dog-to-dog transmission via bite wounds. Infected dogs may show clinical signs including by fever, anorexia, depression, pallor, splenomegaly, and a bounding pulse, which is mainly caused by moderate to severe hemolytic anemia [4]. However, canine babesiosis relatively rare in healthy, spleen-intact adult dogs in Korea. First canine babesiosis caused by *B. gibsoni* has been reported at 1962 in Korea [15] and never been reported babesiosis by *B. canis*, to date. In Korea, canine babesiosis is more common in American Pit Bull Terrier [10].

Canine babesiosis is a tick-borne disease. *Rhipicephalus*



**Fig 2.** MRI images of this case. Identified diffuse lesion with irregular margin shape change of high signal (white arrow) at cerebellum (white bold arrow) on sagittal T2 flair image (A) and lesion of high signal (white circle) on transverse T2 flair image at cerebellum (B).

*sanguineus* and *Dermacentor variabilis* are the most common known arthropod vectors in canine babesiosis [6]. Direct transmission can also occur following blood transfusion or iatrogenic inoculation with contaminated needles or surgical instruments [6]. In certain breeds (e.g. in American Staffordshire and American pit bull terriers), direct dog-to-dog transmission *via* dog bites is more common means of transmission in *B. gibsoni* [10].

Definitive diagnostic method for canine babesiosis is identification of organisms in erythrocytes on stained blood smears, although organisms may be often difficult to see in samples collected from dogs with chronic babesiosis or asymptomatic carriers. Molecular diagnosis using PCR and serological diagnosis using fluorescent antibody are also available to confirm the diagnosis and to identify the species of *Babesia* [6].

Goals of treatment for babesiosis are to reduce parasitemia by chemotherapy using imidocarb dipropionate, atovaquone with azithromycin and diminazene aceturate and to lessen clinical signs using blood transfusion and supportive cares [6]. *B. gibsoni* is considered more difficult to treat than *B. canis* [6]. Imidocarb dipropionate is not considered effective for treating *B. gibsoni* [6].

Diminazene aceturate is 4,4'-(diazooamino) dibenzaminediaceturate and is used in tropical countries for the treatment of animal trypanosomiasis and babesiosis, usually as an intramuscular injection of 3~5 mg/kg [9, 12]. There are few acute toxicity data available with diminazene in dogs and other animals [2]. Mouse fed with oral dose of 1,500 mg/kg diminazene showed signs of toxicity including death, increased spontaneous activity, tactile hyperesthesia and uncoordinated gait. Brain damage associated with diminazene toxicity has been reported in asses and dogs [1, 11]. In dogs treated 24~72 h previously with diminazene, acute neurological signs including spastic paralysis, opisthotonos and nystagmus with involuntary running movements were noted in dogs [2]. Extensive hemorrhagic malacia of the brain stem was noted involving the mesencephalon and diencephalon [11]. In donkeys treated with 7 mg/kg of diminazene, some donkeys had developed central nervous system (CNS) effects after 2 days of administration. Some donkeys died whereas some survived after 14~30 days. Post-mortem examination revealed macroscopic and microscopic hemorrhages in the cerebellum [5].

When given 3.5 mg/kg on two consecutive days by the intramuscular route, no signs of toxicity were observed in dogs. One study found signs of toxicity occurred 6-9 consecutive days after given 3.5 mg/kg/day intramuscularly [14]. All the affected animals showed cerebellar lesions characterized by hemorrhages and areas of malacia [14]. Other study with dogs also found lesions in CNS and cerebellum by diminazene toxicity [11]. One recent study reported clinical case of diminazene toxicity related to CNS signs [8]. Although diminazene toxicities were usually associated with overdose, toxicities could occur at the recommended dose [11, 14]. Fur-

thermore, the brain lesions caused by diminazene toxicity may not be differentiated from those from clinical cerebral.

In this case, the dog was administered with one recommended intramuscular dose (3.5 mg/kg) of diminazene for treatment of babesiosis. However, the dog showed acute CNS signs including ataxia, nystagmus and occasional seizures. MRI study revealed cerebellar lesions. Clinical signs were gradually improved after steroid and phenobarbital administration. Based on medical history, MRI study and therapeutic response we tentatively made diagnosis of acute diminazene toxicity in this case. To author's the best knowledge, this is the first case of diminazene toxicity in dogs at the recommended dose having cerebellar lesions proven by advanced diagnostic MRI studies.

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