“Abilene’s Genes”

Ivermectin Toxicosis in an ABCB1-1Δ Polymorphism Affected Australian Shepherd

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Introduction

Ivermectin toxicosis, though commonly studied and widely understood, still stands to have a pertinent place in small and large animal medicine. Toxicosis can occur following an overdose of a small animal ivermectin product, accidental ingestion, or inappropriate administration of a concentration intended for large animal species or an appropriate dosing in an animal effected by the ABCB1-1Δ polymorphism, formally known as MDR-1 allele mutation.[1] Ivermectin belongs to the macrocytic lactone (ML) family and is widely used in human and veterinary medicine as a parasiticide. Ivermectin acts as a gamma-aminobutyric (GABA) agonist causing cell membrane hyperpolarization via increased postsynaptic permeability to chloride ions.[5] Ivermectin also binds the glutamate-gated chloride channels which similarly cause inhibition of excitatory motor neurons.[5] Clinical signs associated with ivermectin overdose include but are not limited to mydriasis, hypersalivation, ataxia, tremors, respiratory failure, obtundation, comas and death.[1,5,6] These clinical signs are attributed to the enhancement of neuronal inhibition.[3,4] Ivermectin's effectiveness as a parasiticide relates to chloride-meditated channels in peripheral synapses being widely distributed throughout invertebrates. In contrast, these similar chloride channels are exclusively located in limited areas in mammals. This explains why invertebrates require such a small dose to reach proficiency. As no specific antidote for ivermectin toxicity has been found, the most important aspect of treatment is timely decontamination and supportive care.[5] Most recently, intravenous lipid therapy has been shown to have some efficacy. [1,5,9,10] The opportunity for ivermectin overdose is commonly seen due to the its reputation, different formulations (in both large and small concentrations), and its effectiveness across large and small animal species.

History and Presentation

Abilene is an approximately 18-month-old, spayed female Australian Shepherd who presented to the MSU-CVM Internal Medicine Service on 9/20/19 for the evaluation of an acute onset of worsening generalized ataxia, diffuse muscle tremors, head pressing, hypersalivation, and hyperreflexia following the possible ingestion of an unknown toxin. The initial clinical signs observed by the owner on 9/18/19 included slight ataxia, and mild vision impairment. Abilene was taken to her primary veterinarian on the 9/19/19 as these clinical signs appeared to progress over the 24-hour period. Results of a complete blood count and serum biochemical analysis demonstrated a mild increase in red blood cell count, hemoglobin, hematocrit, blood urea nitrogen, and glucose. These changes could be attributed to a dehydrated state and a stress response. After initial diagnostics and stabilization with her primary veterinarian, Abilene was transferred to a 24-hour emergency facility that was equipped to provide intensive care and constant neurological monitoring. Upon presentation to the 24-hour clinic, Abilene condition had progressed and she was then tachycardiac, depressed, hypersalivating, and displayed diffuse muscle tremors. Abilene was administered 400 mg of methocarbamol intravenously, 1 mg/kg cyproheptadine rectally, and 0.02 mg/kg of atropine intravenously. Thereafter, Abilene was started on intravenous fluids, 10 mg/kg/hr constant rate infusion of methocarbamol, and 12 mg of cyproheptadine rectally every 8 hours. During her stay at the 24-hour care clinic, Abilene's clinical signs steadily progressed to head pressing and worsening ataxia over the subsequent 12 hours of treatment. Given this progression despite medical management, Abilene was referred to MSU-CVM Internal Medicine service for further investigation of her ailment. Additionally, a significant detail of Abilene's recent history was two days prior to her clinical state, on 9/16/2019, the horses on Abilene's farm were dewormed with an oral commercial ivermectin

paste labeled for large animals. Abilene frequently accompanies the owners to the farm and has a history of eating the horses' feces.

On presentation to MSU-CVM, Abilene's primary physical examination revealed she was alert, hypersalivating, hyper-reflexive with diffuse muscle tremors, and over-responsive (hyper-reactive) to her surroundings. Her vital parameters were within normal limits with a heartrate of 72 beats per minute, a respiratory rate that fluctuated between 36 and 66 breathes per minute, and a rectal temperature of 101.5 F. Her mucous membranes were slightly hyperemic and moist with a capillary refill time of less than two seconds. Cardiothoracic auscultation revealed no crackles, wheezes, murmurs, or arrhythmias, and abdominal palpation did not reveal a pain response or appreciable organomegaly. Neurologic examination revealed Abilene was ataxic; swaying when standing still and frequently knuckling over when stepping. Conscious proprioception was delayed in all four limbs with the forelimbs appearing more effected than the hindlimbs. Abilene's menace response was inconsistent, and her vision was impaired.

Pathophysiology

Ivermectin is the bestselling broad-spectrum antiparasitic in the world.[5] Its commercial availability, variety of concentrations, and cross species efficacy plays into its popularity. Ivermectin is a highly lipophilic drug and is sub-categized into the avermectin family, which resides within the ML family.[1] Avermectins are derived from naturally occurring compounds produced by *Streptomyces avermitilis,* a soil-dwelling fungi actinomycete.[1,4,5] Ivermectin acts directly on the gamma-aminobutyric acid (GABA) chloride channels and the glutamate-gated chlorine channels.[1] GABA is a potent inhibitory amino acid neurotransmitter that increases postsynaptic cell membrane permeability to chloride ions.[5] As ivermectin binds the GABA receptors, chloride rush in leading to cellular hyperpolarization and a subsequent postsynaptic

blockade of nerve impulses within the nervous system.[5] Receptors for both targeted chloride channels are peripherally widespread in invertebrate parasites. Summarized, treatment with ivermectin results in irreversible channel opening and subsequent hyperpolarization that leads to paralysis and death of these target invertebrates.[1]

Toxicity in mammals requires a much higher concentration of ivermectin compared with that observed in invertebrates. This is believed to be attributed to firstly, the limited amounts of GABA receptors in mammals and secondly, the impermeability of the blood-brain barrier (BBB) to ivermectin.[1,5] Maintained by tight junctions between endothelial cells of capillaries, the BBB is responsible for what enters the CNS. Lipid soluble compounds can readily penetrate the BBB by simple diffusion and saturation.[4] A transport system that is responsible for the removal of unwanted compounds is a large glycosylated transmembrane protein pump, P-glycoprotein (P-GP). P-GP is a major determinant of the concentration of lipid soluble drugs that pass through the BBB and into the CNS. When high doses saturate the receptors, or when dysfunctional P-GP is present, unwanted amounts can accumulate within the CNS.[5] When saturated, the capacity for the removal by the P-GP is exceeded. When the P-GP is defective, the capability of removal is diminished and lost. Thus, the P-GP should function to protect the animal by removing the excess amounts of exposure to toxins. Dysfunction of the P-GP has been traced back to a mutated ABCB1-1Δ allele in certain breeds of dogs.[4,5,6,9] The P-GP defect is a naturally occurring autosomal recessive trait in dogs.[4,9] A spontaneous, 4-base pair deletion occurs characterized by a frame shift, generating a mutation in the fourth coding axon of the ABCB1-1Δ allele.[4,9] This causes several premature stop codons rendering the protein deficient.[9] This genetic deficiency results in a nonfunctional, severely shortened protein, losing more than 90% of the gene sequence.[4] Dogs that are homogenous for the mutation can experience neurological effects

after a single dose of less than 0.1 mg/kg of ivermectin.[4] Yet, reported ivermectin doses as low as 0.08 mg/kg has been shown to cause clinical signs in a dog with the ABCB1-1Δ polymorphism.[4] Furthermore, dogs found to not have the ABCD1-1Δ polymorphism have demonstrated toxicosis at lower dosages than the recommended doses.[6] Breeds that may commonly carry the ABCB1-1Δ polymorphism are primarily herding breeds. Breeds in this category include Collies, Australian Shepherds, Shetland Sheepdogs, Old English Sheepdogs, German Shepherds, and English Shepherds.[1,6]

After an overdose of ivermectin, the onset of clinical signs reportedly ranges from 5 hours to 5 days.[4] Peak plasma concentration is reported at 3-5 hours after therapeutic dosing.[1] Ivermectin's long terminal half-life of 80.3 +/- 29.8 hours is attributed to enterohepatic recirculation.[1] Severity of clinical signs in a study of 17 collies were most apparent on days 4-7 post ingestion of ivermectin.[4] Recovery after ivermectin toxicity has been variably reported at 8 days, 19 days, 49 days, or even multiple weeks to months.[4] Full recovery can take up to two months, with vision impairment and depression being the last clinical signs to resolve.[2] Because of the potential for slow progression of clinical signs and a long half-life, close monitoring for clinical signs for an extended amount of time is recommended following ingestion of ivermectin. A retrospective study using the Animal Poison Control Center data reported the most common clinical signs associated with ivermectin toxicity included ataxia, lethargy, mydriasis, hypersalivation, tremors, and recumbency.[6]

The amount of ivermectin consumed and the presence of the gene mutation will dictate the clinical signs experienced. As previously mentioned, clinical signs range from ataxia to a comatose state with respiratory depression. Therefore, treatment considerations with ivermectin toxicosis will range from simple supportive care to intubation with mechanical ventilation.

Comatose animals with absent gag and swallow reflexes are at increased chances of aspiration pneumonia and should be intubated and receive regular oral-pharyngeal care.[5] The same care holds true for manually ventilated animals. If mentally appropriate, multiple doses of activated charcoal (1-2 g/kg every 8 hours for 6 doses) can be given to interrupt the enterohepatic recirculation that the drug undergoes and thereby reducing the half-life.[10] One report summarized those patients who required machinal ventilation due to neuromuscular toxicities were associated with high recovery rates despite potential side effects.[4] Prognosis for complete recovery from ivermectin toxicity is generally considered to be good.[4] However, reports of dogs receiving 5 mg/kg or greater reveal a guarded prognosis for any breed of dog, even those without the gene mutation.[6]

Another important pathophysiological aspect of this case is the elimination rate of ML and their bioavailability once excreted from the large animals with large animal dose concentrations. ML eliminated in feces serves as a potential source of exposure to small animals. In one study, ivermectin concentrations in horse feces were monitored after horses were treated with a manufacturer's recommended therapeutic dosage. Approximately 90% of the total drug is excreted in feces four days post-treatment.[7] Peak ivermectin levels of 2.4 mg/kg of feces were measured 2.5 days after initial treatment.[7] To place into perspective the amount of ivermectin available in the feces at 2.5 days, 0.6 kg of feces would need to be ingested by Abilene to obtain a 0.1 mg/kg dose of ivermectin; the toxic dose reported for dogs affected with the ABCB1-1Δ polymorphism. [4] While maximum concentration levels of MLs in the feces are reached at 2.5 days post treatment, fecal concentrations of ivermectin remained above detectable levels for 40 days post treatment.[7] Reportedly, accumulated ingestions, although in small concentrations, can lead to toxicosis.[4] Ivermectin has good bioavailability in both oral and subcutaneous

administration. However, the two routes of administration differ in the fact that gastrointestinal absorption is more rapid than when administered subcutaneously.[4]

Diagnostic Approach and Considerations

After triage and stabilization, a computed tomography (CT) imaging and subsequently a cerebrospinal fluid (CSF) analysis were suggested. Although there was a high suspicion that ivermectin toxicity was the culprit for Abilene's neurologic signs, it was assessed that advanced imaging of Abilene's brain and analysis of her CSF were essential to rule out any physiological abnormalities, neoplastic, infectious, inflammatory, structural, or traumatic etiologies. Abilene was placed under general anesthesia and a CT was performed. The CT scan revealed no significant abnormalities, therefore prompting the CSF tap. The CSF fluid analysis revealed no cytological abnormalities.

A diagnostic test that was not performed was testing for the presence of ivermectin in Abilene's plasma. This test would have given confirmation of the presence of ivermectin but would not have provided additional information such as the amount ingested. Additionally, plasma levels are less relevant, as they do not correlate with the ivermectin concentrations within the brain concentration which more directly parallels the clinical presentation of the animal.[4,9] Therefore, given Abilene's presentation and history of access to large animal ivermectin, evaluation of the ivermectin concentration in her plasma was not performed. A benefit of plasma concentrations not utilized in this case is the use of serial ivermectin plasma concentrations to evaluate the effectiveness of ILE therapy.[1]

Before discharge and following improvement in Abilene's clinical signs, samples were collected for a send-out genotyping test used to establish the presence of the ABCB1-1Δ polymorphism.

Treatment and Management

Shortly after the CT and CSF tap were completely, intravenous lipid emulsion (ILE) therapy was initiated. The doses and administration method used in veterinary medicine is patterned after human medicine's dose and administration practices. In cases of a neurotoxicant, a bolus of 1.5 ml/kg is given in 5-10 minutes followed by a continuous infusion of 0.25 ml/kg/min in 30-60 minutes, or 4 mg/kg in 4 hours to minimize the risk of fluid overload.[10] A 20% fat emulsion was administered intravenously to Abilene with an initial bolus of 20 ml over 5 minutes, then as a constant rate infusion of 200 ml/kg/hr for a total of 500 ml through a peripheral cephalic catheter. Abilene was then maintained on intravenous fluid therapy at 30 ml/kg/hr. ILE therapy was first introduced to human medicine for parenteral nutrition and later used to treat cardiotoxicity of local lipid-soluble anesthetic overdose.[10] ILE are composed of natural triglycerides, the most commonly used formulation is composed of a long chain soybean oil-based emulsion.[10] They are an isotonic solution with marketed concentration ranging from 10 to 30%, while mainly 20% has been the documented antidote.[8] The exact mechanism of action is still not completely understood.[1,8,10] A proposed mechanism of action is the large lipid fraction of the emulsion in peripheral blood will bind to lipophilic substances creating a "lipid sink".[1,8,10] This "lipid sink" reduces the circulating concentration of the lipophilic drug or toxicant, therefore reducing its toxic effects and subsequent consequences. The lipophilic drug or toxicant is then cleared from the blood as it is metabolized in the liver and muscular tissue.[10]

Before CSF fluid analysis results were finalized, Abilene was started on 11.5 mg/kg of clindamycin orally every 12 hours and 11.5 mg/kg of doxycycline orally every 24 hours. These antibiotics were instituted to combat the possibility of an infectious cause of Abilene's clinical, such as protozoan parasite or tick-borne diseases. Abilene's attitude, neurological status and respiration rate and quality were monitored hourly during initial ILE therapy. Side effects generally reported with ILE include gastrointestinal upset coupled with pancreatitis due to the iatrogenic hyperlipidemia, and hypersensitivity reactions due to the soybean oil constitutes.[1] ILE has also been associated with immune-suppression, proinflammatory effects, pro-oxidant effects, phlebitis, lipid embolization, lipid keratopathy, and negative hemodynamic effects.[10]

Abilene's clinical signs and neurologic status slowly improved over the next 12 hours. Improvement from presentation was noted as she became more aware of her surroundings and became less ataxic. She continued to head press while in her cage and persistently displayed muscle tremors. With her improved clinical signs following the first ILE treatment, it was decided to administer more ILE therapy. Abilene went on to receive additional 1000 ml of intravenous lipids on her second day of hospitalization following the first 500 ml on the day of presentation; totaling her ILE amount to 1500 ml within the first 24 hours at MSU CVM. Abilene's maintenance fluids, clindamycin and doxycycline were maintained during her hospitalization.

By the completion of the ILE therapy, Abilene was no longer displaying muscle tremors or head pressing in her cage. Slight vision impairment and slight ataxia were still appreciated but found to be much improved. She became brighter and more alert with appropriate responses to environmental stimulations each day throughout her 4-day hospitalization at MSU CVM.

Case Outcome

At the time of discharge from MSU CVM, Abilene was showing interest in her surroundings, and was more playful. Although her menace had not fully returned, her vision was significantly improved. She was still slightly ataxic, and her conscious proprioceptive deficits were still present but markedly improved**.** Abilene was discharged with recommendations of cage rest when unsupervised, reducing contact with large animals until fully recovered, hand feeding until she was able to locate her food and water independently, and monitoring for worsening clinical signs. Adverse effects associated with ILE therapy were also discussed with the owners and they were instructed to monitor for any of the associated clinical signs.

Abilene was reevaluated seven days after discharge. At this recheck, Abilene was bright, alert and appropriately responsive to her surroundings. Her vital parameters and physical examination findings were found to be within normal limits. Abilene's neurological exam revealed all cranial nerves, spinal reflexes, and conscious proprioception were within normal limits. No pain was elicited on palpation of the cervical, thoracic, or lumbar spinal segments, and normal tone was noted in the forelimbs and hindlimbs. No adverse effects associated with the ILE therapy were seen by Abilene’s owner. Given Abilene's improved status, returning to normal activity and exercise was approved. She was discharged that afternoon with no recheck indicated unless the owners noticed the return of any clinical signs.

The results of her genetic testing for the ABCB1-1Δ polymorphism revealed that Abilene possessed the autosomal recessive trait, thus confirming that Abilene is affected with the mutated allele causing deficient P-GP transportation and leading to the increased hypersensitivity to ivermectin. The owners were informed of the results, and of the drugs that should be avoided or used with great caution in the further.

Conclusion

When proper dosages and concentrations are used, ivermectin is a safe and effective drug across multiple species.[5] Overall caution should be used when utilized in an off-label manner due to the fact as previously discussed and reported, some animals can become symptomatic at dosages or accumulated doses not previously considered to cause clinical problems. For at risk breeds, testing prior to administration of higher doses of ivermectin or P-GP substrates is prudent.[6] Educating owners of clinical signs associated with an overdose in patients that are receiving off-label dosages of ivermectin should be of standard practice. It is also important for clients to be made aware of the risk of ivermectin formulations used for large animal species pose as potential a source that can affect their small animals, such as in Abilene's case.

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