Azathioprine-induced bone marrow toxicity in four dogs

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Azathioprine is a thiopurine antimetabolite that is commonly used in veterinary medicine as an immunosuppressive agent (1). In combination with glucocorticoids, it is often used for the management of immune-mediated diseases, such as immune-mediated thrombocytopenia and immune-mediated hemolytic anemia. Antimetabolites compete with endogenous purines, causing the formation of nonsense sequences in RNA and DNA, which ultimately leads to a fall in lymphocyte number and impaired lymphocyte function. Azathioprine is metabolized by the liver and converted to 6-mercaptopurine and 6-thiosinic acid, the active form (1,2). Cyclophosphamide has traditionally been the immunosuppressive drug of choice in veterinary medicine; however, its use is commonly associated with myelosuppression and sterile hemorrhagic cystitis (7). Azathioprine is being used with increasing frequency, as it is reported to have a more rapid onset of action than cyclophosphamide (3), and is believed to be less toxic (1). Azathioprine toxicity is rarely described in the veterinary literature. The purpose of this paper is to describe 4 dogs with bone marrow suppression associated with the use of azathioprine.

Case 1

A 5-year-old, neutered male, cocker spaniel was diagnosed with immune-mediated thrombocytopenia. It was treated initially with daily prednisone (Apo-Prednisone, Apotex, Toronto, Ontario) (2 mg/kg body weight [BW]) and azathioprine (Imuran, Burroughs Wellcome, Kirkland, Quebec) (2 mg/kg BW); the prednisone dose was then tapered to 0.75 mg/kg BW as the dog responded. Complete blood counts (CBC) were performed every 2 wk to monitor platelet and leukocyte numbers. Approximately 12 wk after azathioprine therapy was initiated, a CBC revealed a decreased platelet count of 80×10^9/L and a normal neutrophil count of 6.8×10^9/L. The red blood cell count was normal. Recurrence of immune-mediated thrombocytopenia was suspected and the prednisone dosage was increased from 0.75 mg/kg BW daily to 2 mg/kg BW daily. The azathioprine dosage remained at 2 mg/kg BW daily. A CBC was evaluated 4 d later and abnormalities included a platelet count of 53×10^9/L and a falling neutrophil count of 4.1×10^9/L. A tentative diagnosis of azathioprine-induced bone marrow suppression was made, and azathioprine was discontinued. A CBC performed 10 d after withdrawl of azathioprine revealed a normal platelet count of 194×10^9/L and a slighlty elevated neutrophil count of 18.7×10^9/L.

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Case 2

A 7-year-old, female boxer was diagnosed with a severe nonregenerative anemia. Based on spherocytosis and splenomegaly, a tentative diagnosis of immune-mediated hemolytic anemia was made. The dog was treated with prednisone (2 mg/kg BW) daily, but failed to respond. When treatment with both prednisone (2 mg/kg BW) daily and azathioprine (2 mg/kg BW) daily was initiated, an increase in hematocrit was noted. The dog continued to improve over the next 3 mo, and the prednisone dosage was gradually decreased. Futher rechecks were declined by the owner at that time. Sixteen weeks after therapy had been initiated, the dog was presented for evaluation of lethargy. Therapy at that time included only azathioprine at 2 mg/kg BW daily; the prednisone therapy had been discontinued by the owner. Abnormalities noted on CBC included pancytopenia with a hematocrit of 0.19 L/L, total nucleated cell count of 1.6×10^9/L, neutrophil count of 0.83×10^9/L, and platelet count of 26×10^9/L. A tentative diagnosis of azathioprine-induced bone marrow suppression was made, and the azathioprine therapy was discontinued. Prednisone therapy was reinstituted at a dosage of 2 mg/kg BW daily. A CBC performed 1 wk later revealed a nucleated cell count of 4.2×10^9/L and a neutrophil count of 3.36×10^9/L, with no improvement in the other cell types. Subsequent CBCs revealed a gradual improvement of all cell types.

Case 3

A 7-year-old, neutered male, soft-coated wheaten terrier was treated with prednisone (2 mg/kg BW daily) for immune-mediated arthritis of the temporomandibular joint. Azathioprine (2 mg/kg BW daily) was added 3 wk after diagnosis, because the dog failed to respond to the prednisone alone. Four weeks after azathioprine therapy had been initiated, the dog was presented for evaluation of lethargy and inappetance. A CBC revealed pancytopenia with a hematocrit of 0.125 L/L, total nucleated cell count of 0.8×10^9/L, neutrophil count of 0.36×10^9/L, and platelet count of 13×10^9/L. A tentative diagnosis of azathioprine-induced bone marrow toxicity was made and azathioprine was discontinued. A recheck CBC done 6 d after discontinuation of the azathioprine revealed no improvement of the pancytopenia, and a bone marrow aspirate and core biopsy sample were obtained. The dog was treated with a transfusion of fresh whole blood at that time. Cytologic examination of the aspirate revealed a hypocellular marrow with very few early myeloid and erythroid cells and no megakaryocytes. Histological findings were similar to those found on cytological examination. These findings supported a diagnosis of marked bone marrow hypoplasia with mild myelofibrosis. A CBC performed 14 d after discontinuation of the azathioprine revealed an increased total nucleated cell count (3×10^9/L), increased neutrophil count (1.7×10^9/L), and an increased platelet count (20×10^9/L). The hematocrit was increased to 0.24 L/L; however, the dog had received a transfusion of

fresh whole blood 8 d previously. A CBC performed 3 wk following the discontinuation of azathioprine revealed a normal total nucleated cell count, with slight improvement of the other cell types. Subsequent CBCs revealed the gradual return of all cell types to normal values approximately 8 wk after discontinuation of the azathioprine.

Case 4

An 8-year-old, spayed, female cocker spaniel was treated for immune-mediated thrombocytopenia (ITP) with prednisone (2 mg/kg BW) daily. One week after diagnosis, the platelet count had failed to improve and azathioprine (2 mg/kg BW) daily was added. The dog responded well and was maintained on a decreased dosage of prednisone (0.5 mg/kg BW every other day) and azathioprine (0.5 mg/kg BW every other day) for approximately 12 mo. At that time, the dog appeared to be normal; however, a routine CBC revealed thrombocytopenia (39×10^9/L) and a recurrence of ITP was suspected. The dosage of azathioprine was increased from 0.5 mg/kg BW to 1 mg/kg BW every other day. The CBCs performed 1, 2, and 3 wk later revealed persistent thrombocytopenia, with all other cell types within normal limits. The azathioprine dosage was then increased to 2 mg/kg BW daily and prednisone dosage to 2 mg/kg BW daily. A CBC performed 4 wk later revealed neutropenia (0.22×10^9/L) and thrombocytopenia (3×10^9/L). A tentative diagnosis of azathioprine-induced bone marrow toxicity was made and the azathioprine was discontinued. A bone marrow aspirate was taken, and cytological findings included megakaryocyte hypoplasia, and ineffective erythropoiesis and myelopoiesis. The neutropenia resolved within 2 wk of discontinuing the azathioprine therapy, and the thrombocytopenia resolved within 3 wk.

In all 4 dogs, the azathioprine therapy was initiated for the treatment of immune-mediated disease. Diagnosis of azathioprine-induced bone marrow toxicity was made upon observation of leukopenia ($<3.9 \times 10^9$neutrophils/L), thrombocytopenia ($<145 \times 10^9$platelets/L) and, in some cases, anemia ($<5.6 \times 10^{12}$red blood cells/L, hematocrit <0.38 L/L), followed by recovery upon withdrawal of the drug.

Although the principle side effect of azathioprine cited in the dog and cat is bone marrow suppression, manifested primarily as leukopenia and thrombocytopenia (1,3), and in some cases anemia, this effect has rarely been documented. Other toxic effects that have been reported in the dog include pancreatitis and hepatotoxicity (3,1). The development of azathioprine-induced bone marrow suppression in humans has been associated with homozygosity for allelles encoding thiopurine methyltransferase (TPMT) of low activity (4,6). The enzyme TPMT plays an important role in azathioprine catabolism. If TPMT levels are low, metabolites of azathioprine may accumulate within cells. The 6-thioguanine nucleotides (6-TGN) are cytotoxic metabolites of azathioprine catabolism. Intracellular accumulation of 6-TGN and the incorporation of 6-TGN into nucleic acids of hematopoietic progenitor cells are thought to be the cause of bone marrow failure. A human study revealed low TPMT activities and abnormally high 6-TGN concentrations in 5 patients with myelosuppression and concurrent azathioprine therapy (4). It is possible that a similar mechanism for azathioprine-induced bone marrow toxicity exists in the dog and cat.

The presumptive diagnosis of azathioprine-induced bone marrow toxicity is based on observation of leukopenia and/or thrombocytopenia coincidental with the administration of the drug. Nonregenerative anemia may also develop, as was seen in 2 of the 4 dogs described here. In the remaining dogs, azathioprine-induced myelosuppression was diagnosed very early, the drug was withdrawn, and anemia did not develop. Bone marrow analysis will reveal a hypocellular marrow, as is seen with toxicity from estrogens, phenylbutazone, and chemotherapeutic agents (5). The bone marrow analyses done in dogs 3 and 4 both revealed a decrease in all cell types. If the disease process is diagnosed at an early stage, the red cell types may appear normal. If the condition is not recognized, aplastic anemia and myelofibrosis may develop. In humans, the severity of the myelosuppression is related to the dose and duration of the azathioprine therapy. In a study of 5 human patients with azathioprine-induced bone marrow suppression, the duration of treatment ranged from 21 to 70 d (mean 28 d) (4). In the dogs reported here, the duration of therapy ranged from 1 to 12 mo. A case report of a dog with acute pancreatitis and bone marrow suppression, subsequent to the administration of azathioprine, was reported to have decreased neutrophil counts for 5 mo (3).

The treatment for azathioprine-induced bone marrow toxicity is supportive. Azathioprine therapy should be discontinued permanently. The marrow toxicity is usually reversible; however, in humans, leukopenia may persist for up to 7 d. In the cases reviewed here, neutrophil counts returned to normal within 1 to 3 wk after the azathioprine had been withdrawn, and platelet counts within 3 to 6 wk. The prognosis for bone marrow recovery is relatively good. If, however, the condition is not recognized, severe myelofibrosis may develop, with an attendant guarded prognosis for recovery.

Thus, during azathioprine therapy, complete blood and platelet counts should be monitored every 1 to 2 wk initially, and then once monthly while the dog is on maintenance therapy, to allow early detection of potential myelosuppression. CVJ

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