

Azathioprine

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Azathioprine has been used as an immunosuppressive agent in dogs for over 50 years. The drug was initially primarily used in studies that utilized dogs as a model for investigations of organ transplantation and the effects of immunosuppression on various body systems. Within a few years, azathioprine was also being used to treat naturally occurring diseases in dogs. Despite almost half a century of cumulative clinical and research experience on the use of azathioprine in dogs, however, there have been remarkably few studies that actually elucidate the precise effects that azathioprine has on the canine immune system. Most of our understanding of the mechanism of action of azathioprine in dogs is extrapolated from work in other species.

Azathioprine is a prodrug for the active metabolite 6-mercaptopurine, and the primary mechanism of action was long believed to be inhibition of the synthesis of the purines adenine and guanine by blockage of enzymes such as amidophosphoribosyltransferase, with resultant production of nonfunctional nucleic acid strands. Disruption of *de novo* purine synthesis inhibits DNA and RNA synthesis, thereby inhibiting the proliferation of fast-growing cells such as lymphocytes. Lymphocytes are particularly susceptible to the effects of inhibition of *de novo* purine synthesis, because they are relatively lacking in the alternative pathway of purine synthesis, the purine “salvage” pathway, in which nucleotides are re-synthesized from nucleotide degradation products. In the past few decades, however, multiple other mechanisms of action mediated by various azathioprine metabolites have been proposed, including blockage of T cell activation and stimulation of T cell apoptosis. Azathioprine has long been reported to be more effective against T cell function than B cell function, although strong evidence supporting this is lacking, and recent work in our laboratory demonstrated that azathioprine inhibits both B and T cell proliferation.

One of the key enzymes involved in azathioprine metabolism and inactivation is thiopurine methyltransferase (TPMT). Individual human patients (about one in 300 people) inherit a marked deficiency in the TPMT enzyme that renders them highly susceptible to azathioprine toxicity, particularly life-threatening bone marrow suppression. Interestingly, cats have also been shown to have a marked deficiency in TPMT enzyme activity, which may explain why azathioprine causes marked myelosuppression in cats at standard canine doses. Although the use of azathioprine at a very reduced dose rates has previously been published in cats, given the narrow margin for safety it is probably wisest to recommend that azathioprine never be used in cats at any dose, especially considering the availability of other immunosuppressive agents that appear to be much safer in cats, such as chlorambucil and cyclosporine. Although TPMT expression in dogs is widely variable, severe deficiencies in enzyme activity of the magnitude seen in cats and some people have not been commonly reported, and TPMT deficiency does not typically appear to be associated with the severe drug toxicities sometimes seen in dogs. In people, despite known variabilities in drug metabolism, pharmacokinetic monitoring of azathioprine has not become established, although several recent papers have suggested that monitoring of drug metabolites such as 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MeMP) may help predict drug efficacy and toxicity.

The standard azathioprine starting dose in dogs is 2 mg/kg orally once daily. This dose is usually well-tolerated and, although gastrointestinal side effects such as nausea, anorexia, vomiting and diarrhea are occasionally reported, they are typically mild and self-limiting. Although, in dogs, marked myelosuppression is uncommon, chronic azathioprine usage sometimes causes mild to moderate poorly regenerative anemia. Since anemia is a possible outcome in dogs receiving azathioprine, and is typically very well tolerated (that is, sub-clinical), mild to moderate anemia alone should not be mistaken as evidence of either drug overdose or treatment failure. Azathioprine can also cause profound myelosuppression or severe hepatotoxicity in some dogs. Marked myelosuppression and hepatotoxicity appear to be idiosyncratic non-dose-dependent reactions (Type B reactions), and are typically reversible if the problem is recognized early enough and azathioprine is discontinued. Severe myelosuppression is uncommon, but hepatotoxicity (typically characterized by a rise in reversible rise in ALT in the absence of clinical signs) occurs in about 15% of dogs. Hepatotoxicity may be more common in German Shepherds. Hepatotoxicity usually develops in the first few weeks of therapy and, if the drug is well-tolerated for

the first 2 to 4 weeks of therapy, it tends to be well-tolerated long-term. Myelosuppression, in contrast, can be more delayed. Anecdotally, some veterinarians believe that hepatotoxicity is more common with the generic forms of azathioprine compared to the proprietary product, but this observation may be limited to past reported individual incidents with generic azathioprine from dubious (overseas) sources. Complete blood counts and serum biochemistry panels (especially ALT) should be monitored regularly during initial azathioprine therapy. Several individual case reports have also reported pancreatitis in dogs receiving azathioprine, but cause and effect has not been established.

Azathioprine has, over the years, become well-established as an “add on” immunosuppressive agent to be considered for the treatment of many different immune-mediated and inflammatory conditions when glucocorticoids alone are ineffective or poorly tolerated, including immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, inflammatory bowel disease, chronic hepatitis, glomerulonephritis, immune-mediated polyarthritis, myasthenia gravis, non-infectious meningoencephalitis, immune-mediated skin diseases, and anal furunculosis. Despite decades of azathioprine usage, evidence supporting immunosuppressive efficacy for many of these common diseases is remarkably limited. One recent study in a large number of dogs showed that one year survival in dogs with IMHA on azathioprine and prednisolone was only slightly better than outcomes in dogs on prednisolone alone. Interestingly, because (despite a relative paucity of evidence) azathioprine has commonly been recommended as the standard immunosuppressive drug of choice for many conditions, the efficacy of newer drugs for the treatment of these conditions is sometimes compared to a parallel group receiving azathioprine. One perceived “limitation” of azathioprine compared to other immunosuppressive agents, that it can take many weeks or even months to exert its effects, is based on limited and dated data derived predominantly in humans. In my experience, azathioprine in a clinical setting exerts its immunosuppressive effects in dogs about as rapidly as most other comparable agents, and recent research in our laboratory also demonstrated inhibition of canine lymphocyte proliferation within 2 weeks of commencing azathioprine.

Compared to most other immunosuppressive agents, azathioprine is relatively inexpensive, which is an important consideration with long-term immunosuppressive therapy, especially in large dogs. While the proprietary product (Imuran[®] or Azasan[®]) typically still costs over \$5 per 50 mg tablet, the generic equivalent can be obtained for less than \$1 a tablet. The smallest tablet size is 50 mg (although tablet scoring permits a 25 mg dose), which can present dosing problems in small (under 20 lb) dogs.