Pharmacokinetics of azathioprine following single-dose intravenous and oral administration and effects of azathioprine following chronic oral administration in horses

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Objective—To determine pharmacokinetics of azathioprine (AZA) and clinical, hematologic, and serologic effects of IV and oral administration of AZA in horses.

Animals—6 horses.

Procedure—In study phase 1, a single dose of AZA was administered IV (1.5 mg/kg) or orally (3.0 mg/kg) to 6 horses, with at least 1 week between treatments. Blood samples were collected for AZA and 6-mercaptopurine (6-MP) analysis 1 hour before and at predetermined time points up to 4 hours after AZA administration. In study phase 2, AZA was administered orally (3 mg/kg) every 24 hours for 30 days and then every 48 hours for 30 days. Throughout study phase 2, blood samples were collected for CBC determination and serum biochemical analysis.

Results—Plasma concentrations of AZA and its metabolite, 6-MP, decreased rapidly from plasma following IV administration of AZA, consistent with the short mean elimination half-life of 1.8 minutes. Oral bioavailability of AZA was low, ranging from 1% to 7%. No horses had abnormalities on CBC determination or serum biochemical analysis, other than 1 horse that was lymphopenic on day 5 and 26 of daily treatment. This horse developed facial alopecia from which 1 colony of a Trichophyton sp was cultured; alopecia resolved within 1 month after the study ended.

Conclusions and Clinical Relevance—Overall, no adverse effects were observed with long-term oral administration of AZA to horses, although 1 horse did have possible evidence of immunosuppression with chronic treatment. Further investigation of the clinical efficacy of AZA in the treatment of autoimmune diseases in horses is warranted. (Am J Vet Res 2005;66:1578–1583)

Autoimmune diseases, such as hemolytic anemia, thrombocytopenia, and pemphigus foliaceus, can present veterinarians with a therapeutic dilemma when they occur in horses. In other species, such as humans and dogs, a number of immunosuppressive agents have been shown to be effective and relatively safe for the treatment of autoimmune disease. Unfortunately, corticosteroids and injectable gold salts are the only immunosuppressive treatments that have been used with any frequency in horses. Although these agents are often effective, the high doses of corticosteroids necessary for successful treatment are associated with an increased risk of adverse effects, including steroid hepatopathy, laminitis, and iatrogenic hyperadrenocorticism. Therefore, an investigation of additional immunosuppressive therapeutic agents to treat autoimmune diseases in horses is needed.

Azathioprine (AZA) is an imidazole derivative of 6-mercaptopurine (6-MP) that is commonly used in humans and dogs to treat autoimmune diseases and to prevent rejection of organ transplants. It is a pro-drug that is converted in the blood, liver, and other organs to 6-MP, which is subsequently metabolized by hypoxanthine-guanine phosphoribosyltransferase into 6-thioguanine monophosphate. This metabolic route ultimately leads to the formation of pharmacologically active 6-thioguanine nucleotides (6-TGNs), which act as fraudulent nucleotides disrupting DNA and RNA synthesis, thereby inhibiting cellular proliferation and reducing antibody production and lymphocyte numbers. Elimination of the drug from the body is dependent upon metabolism of 6-MP to inactive metabolites by either xanthine oxidase or thiopurine methyltransferase (TPMT). This latter enzyme appears to be critical, as genetically determined deficiencies of TPMT in humans correlate well with the incidence of myelosuppression, an adverse effect also observed in dogs and cats. Other reported adverse effects in small animals include infection, hepatitis, pancreatitis, diarrhea, vomiting, and alopecia.

Several reports indicate that AZA may be an effective alternative immunosuppressive agent in horses when corticosteroid administration is unsuccessful. The clinical experience of one of the authors (SDW) also supports AZA as effective in the treatment of horses with autoimmune diseases that are intolerant of high doses of corticosteroids or in the treatment of horses with autoimmune diseases that are refractory to corticosteroid treatment alone. Although AZA appears to be a promising immunosuppressive agent in horses, its basic pharmacokinetic parameters and the tolerance of horses to the drug during chronic administration have yet to be established. The objectives of the study reported here were to determine the pharmacokinetics
of AZA following oral and IV administration in horses and to investigate the incidence of adverse effects occurring during chronic oral administration of the drug.

Materials and Methods

Animals—Six horses, 3 mares and 3 geldings, ranging in age from 5 to 17 years old and in weight from 536 kg to 616 kg, were used in this study. Breeds represented were Thoroughbreds (n = 3), Quarter Horses (2), and a cross-bred (1). Horses were judged to be healthy on the basis of findings on physical examination, CBC determination, and serum biochemical analysis. Horses were housed in dry lots or stalls, had free access to water, and were fed an alfalfa and oat hay mixture twice a day. The experimental protocol was approved by and followed the guidelines of The University of California Institutional Animal Care and Use Committee.

Pharmacokinetics of AZA (study phase 1)—In the first phase of the study, AZA was administered once IV (1.5 mg/kg) and once orally (3 mg/kg) to each of the 6 horses by use of a balanced crossover design with at least 1 week between treatments. The oral dose was the highest oral dose previously reported for horses.10 The IV dose was chosen to approximate the only report of parenteral administration of AZA in horses (1.1 mg/kg, IM)9 and as a precautionary halving of the oral dose in response to the low TPMT activity previously reported for horses.11 For IV administrations, AZA was given as a short infusion over 5 minutes through a 14-gauge jugular vein catheter. Blood samples were collected via a contralateral jugular venipuncture 1 hour before and 15, 30, and 45 minutes and 60, 90, and 120 minutes after beginning IV administration of drug. For oral administration of drug, AZA tablets were mixed with water and corn syrup and were given to horses by oral gavage with a dose syringe. Blood samples were collected by jugular venipuncture 1 hour before and 15, 30, and 45 minutes and 1, 1.5, 2, 3, and 4 hours after oral administration of AZA. All blood samples were placed in potassium EDTA tubes, mixed 6 times, and immersed in an ice-water slurry. Blood samples were centrifuged at 4°C within 15 minutes of collection, and the plasma was immediately collected and frozen at −20°C until analyzed by high-performance liquid chromatography. Blood samples were also collected for CBC determination 1 day before and 7 days after each drug administration.

Effects of chronic AZA administration (study phase 2)—In the second phase of the study, AZA (3 mg/kg) was administered orally every 24 hours for 30 days and then every 48 hours for an additional 30 days to the same 6 horses that were used in study phase 1. As noted previously, the oral dose was the highest oral dose previously reported for horses; however, in those reports, it was given at that dose for 7 days or 17 days.1 During study phase 2, blood samples were collected by jugular venipuncture from horses on days 0, 5, 9, 12, 19, 26, 33, 40, 47, 54, 61, and 68 for CBC determination and on days 5, 19, 33, 47, 61, and 68 for serum biochemical analysis. Physical examinations, which included measurements of rectal temperature and pulse and respiration rates, were performed 3 times a week for the 60 days of study phase 2. In particular, horses were examined for any signs that could be consistent with AZA-related adverse effects, such as infection, abnormal bleeding, pale mucous membranes, gastrointestinal tract distress, icterus, or alopecia.

Determination of TPMT activity in RBCs—To measure TPMT activity in RBCs, blood samples were collected from all horses prior to any AZA administration. Samples were collected by jugular venipuncture into evacuated glass tubes containing sodium heparin and shipped overnight chilled, but not frozen, to the Mayo Clinic in Rochester, Minn. The TPMT activity values, reported in units of activity per milliliter of RBCs (U/mL of RBCs), were determined by use of a previously described protocol.13

Determination of plasma AZA and 6-MP concentrations—All reagents were analytic grade unless otherwise indicated. Water was doubly distilled and deionized. To prepare the stock solutions, 4 mg of AZA was dissolved in 50 mL of methanol with sonication; 4 mg of 6-MP was dissolved in 2 mL of sodium hydroxide (0.1M) and then taken to 50 mL in water; and 4 mg of 2-aminomercaptopurine (6-AMP), the internal standard, was treated in the same manner as 6-MP. The saturated solution of EDTA (2.5 g of EDTA mixed into 25 mL of water with vortexing) was prepared the previous day. The 0.2% solution of glacial acetic acid was prepared the day of use. Azathioprine and its major metabolite 6-MP were extracted and analyzed by use of a method modified from Van Os et al.12 One hundred microliters of saturated EDTA and 100 μL of the 6-AMP internal standard solution (equivalent to 400 ng of 6-AMP) were added to 1-mL aliquots of plasma. Sample volumes were then adjusted to 3 mL with water. Prior to solid-phase extraction, C18 cartridges were conditioned with 3 mL of methanol and 5 mL of 0.2% acetic acid in order. Samples were applied to the prepared cartridges and allowed to pass under gravity, after which the cartridges were washed with 3 mL of 0.2% acetic acid and dried for 2 minutes under vacuum at 25 psi of pressure. Following elution with 3 mL of methanol, samples were dried under a stream of nitrogen (40°C) and redissolved in 160 μL of a 5:95 ratio of acetonitrile-to-KH2PO4 (10mM; pH, 3.20). Resulting extracts were transferred to vials for immediate analysis by use of high-performance liquid chromatography with a UV detector.

Plasma concentrations of AZA and 6-MP were determined by use of a high-performance liquid chromatography system14 with UV detection at 323 nm. Sample volumes of 60 μL were injected and separated across a C18 column (350 × 4.6 mm, ID, 10 μm)3 under a 1 mL/min flow rate at a constant temperature of 25°C. Separation was achieved by use of a gradient program, where mobile phase A was 10mM KH2PO4, (pH, 3.20) and mobile phase B was an 8:2 ratio of acetonitrile-to-10mM KH2PO4 (pH, 3.20). Initial gradient conditions began at 4% mobile phase B and were held for 8 minutes. During the next 10 minutes, mobile phase B increased from 4% to 50%. After a 5-minute hold at 50%, mobile phase B decreased 50% in 3 minutes back to the initial conditions. The column was allowed to equilibrate for 5 minutes before the next injection.

Quantification of AZA and 6-MP sample responses was determined as ratios with 6-MP against 5-point extracted standard calibration curves (R2 ≥ 0.995). Prepared in negative control equine plasma and extracted alongside the samples, calibration curve standards contained AZA and 6-MP. Calibration curves ranged from 5 to 500 ng/mL and from 500 to 10,000 ng/mL. Limits of detection were determined to be 2 or 5 ng/mL, dependent on individual run, with limits of quantitation (LOQs) of 5 ng/mL for both analytes. Quality control standards, also prepared in negative control plasma and extracted alongside samples, had mean measured concentrations (n = 5) of 53.26 ng/mL (6.68%, coefficient of variation [CV]) and 2,156.81 ng/mL (0.34%, CV) for AZA standards of 50 and 2,000 ng/mL, respectively. For 6-MP standards of 50 and 2,000 ng/mL, the mean measured concentrations (n = 5) were 51.21 ng/mL (0.52%, CV) and 1,833.29 ng/mL (3.90%, CV), respectively.
Pharmacokinetic analysis—Plasma AZA concentrations were evaluated by use of standard compartmental analysis by use of a software program. The IV infusion data were weighted by the reciprocal of plasma AZA concentrations to reduce the bias in curve-fitting associated with the large difference in magnitude between the highest and lowest plasma AZA concentrations. The appropriate compartmental model was chosen on the basis of the Akaike information criteria. Data from oral administration were analyzed compartmentally for each individual horse if the AZA concentrations of at least 4 plasma samples exceeded the LOQ of the assay. Data were not analyzed if this criterion was not met. Standard compartmental equations were used to estimate the pharmacokinetic parameters for each horse after IV infusion and oral AZA administrations. For data obtained following the IV infusion of AZA, the equation \( C_0 = \text{dose} / \text{volume of distribution} \) was then estimated by use of the equation \( AUC_{IV} = C_0 / k_e \), where \( k_e \) is the elimination rate constant, which was determined from the compartmental analysis. The oral bioavailability of AZA was estimated as the ratio of the dose-normalized AUC following oral AZA administration to that associated with IV administration. Whereas mean values were determined for most pharmacokinetic parameters, the harmonic mean was instead calculated for the elimination half-life to give a more accurate estimate of this parameter.

Results

Pharmacokinetics of AZA (study phase 1)—The plasma AZA concentrations that resulted from the IV infusion of AZA were best described by a 1-compartment model, as determined from the Akaike information criteria associated with various models and from visual inspection of the resulting residuals. Pharmacokinetic parameters associated with the IV infusion of AZA (1.5 mg/kg) to 6 horses were determined (Table 1). Following IV administration, plasma concentrations of AZA rapidly declined and were below the LOQ of the assay (3.0 ng/mL) in 3 of 6 horses by 30 minutes after AZA administration. Plasma concentrations of AZA then rapidly declined and were below the LOQ of the assay in 2 of 6 horses by 30 minutes after AZA administration. Consistent with these findings, the elimination half-life (\( t_{1/2} \)) and mean residence times for AZA were short.

A 1-compartment model with first-order absorption also best described the oral disposition of AZA. Pharmacokinetic parameters for AZA following a single oral dose of AZA (3 mg/kg) to 6 horses were determined (Table 2). In all of the study horses, plasma AZA concentrations remained low, although the peak plasma concentrations varied considerably among horses (Table 3). For example, peak plasma concentrations of AZA ranged from 0 to 42 ng/mL. Pharmacokinetic analysis was performed on only 4 of 6 horses, as 2 horses had < 4 quantifiable plasma AZA concentrations. No AZA was detected in the plasma of 1 horse, although 6-MP could be quantified in this horse for 2 hours following administration. In the 4 horses in which pharmacokinetic analysis was performed, the mean ± SD maximal plasma AZA concentration was 29.7 ± 14.4 ng/mL occurring at a mean of 23 ± 2 minutes after AZA administration. By 2 hours after AZA administration, plasma AZA concentrations of all horses were below the LOQ of the assay. Plasma concentrations of 6-MP after oral administration of AZA followed a similar pattern as the parent compound. For example, the peak plasma concentrations of 6-MP ranged from 9.7 to 30.0 ng/mL and occurred between 15 and 60 minutes after the administration of AZA. By 2 hours after AZA administration, the plasma 6-MP concentrations in all horses were at or below the LOQ of the assay. The bioavailability of AZA was poor, ranging from 0% to 7% in the 6 horses.

Effects of chronic AZA administration (study phase 2)—During the 60 days of AZA administration, no clinically important effects were observed on physical examination. During this period, 2 horses had mild lacerations, 1 on the face and the other on the jaw; both healed normally and uneventfully. The horse with the facial laceration also developed partial patchy facial

Table 1—Pharmacokinetic parameters for azathioprine (AZA) following an IV administration of a single dose (1.5 mg/kg) to 6 horses.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Mean or harmonic parameter mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{max} ) (ng/mL)</td>
<td>5,655 ± 2,368 (3,142–8,741)</td>
</tr>
<tr>
<td>AUC (ng·h/mL)</td>
<td>571 ± 262 (307–927)</td>
</tr>
<tr>
<td>( t_{1/2} ) (min)</td>
<td>1.8 ± 0.4 (1.4–2.4)*</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>2.7 ± 0.5 (2.0–3.4)</td>
</tr>
<tr>
<td>Clearance (mL/min/kg)</td>
<td>51 ± 21 (27–82)</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>0.14 ± 0.05 (0.08–0.21)</td>
</tr>
</tbody>
</table>

\( C_{max} \) = Maximum predicted plasma concentration. AUC = Area under the plasma concentration versus time curve. \( t_{1/2} \) = Apparent elimination half-life. MRT = Mean residence time. Vd = Apparent volume of distribution. * = Harmonic mean.
aloepecia. One solitary colony of a *Trichophyton* organism, a species of dermatophyte, was cultured from hair obtained from the lesional areas. Histologic examination of skin biopsy specimens from the face revealed no fungal organisms with a periodic acid-Schiff stain. Most hair follicles were in early anagen (growth phase), indicating that the alopecia was in the process of resolving. The alopecia resolved within 30 days of the termination of the study.

During the 60 days of AZA administration, no clinically important changes in CBCs or findings on serum biochemical analysis were detected (data not shown). For example, the mean ± SD (n = 6) RBC counts before and after 60 days of AZA administration were 8.2 ± 0.8 X 10⁶ cells/µL and 7.5 ± 0.7 X 10⁶ cells/µL, respectively. In a similar manner, the mean platelet counts (n = 6) were 184,000 ± 31,000 platelets/µL and 176,000 ± 25,000 platelets/µL. Although values for individual horses varied slightly from day to day, none of the horses became anemic (ie, RBC count < 5.5 X 10⁶ cells/µL) or thrombocytopenic (platelet count < 100,000 platelets/µL). The horse with the facial areas of alopecia had 2 lymphopenic episodes ranging from 1.5 to 2.6 U/mL of RBCs, with a mean ± SD of 2.0 and a median of 1.9.

Table 2—Pharmacokinetic parameters for AZA following oral administration of a single dose (3 mg/kg) to 4 horses. *

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₘₐₓ (ng/mL)</td>
<td>28.7 ± 14.4 (16.9–42.6)</td>
</tr>
<tr>
<td>Tₘₐₓ (min)</td>
<td>23 ± 2 (20–25)</td>
</tr>
<tr>
<td>AUC (ng·h/mL)</td>
<td>38 ± 20 (17–57)</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>4 ± 3 (1–7)</td>
</tr>
</tbody>
</table>

*Data are from 4 of 6 horses for which at least 4 plasma AZA concentration determinations of this parameter varying from 15 to 30 minutes.*

Table 3—Plasma AZA and 6-mercaptopurine (6-MP) concentrations following oral administration of a single dose (3 mg/kg) of AZA to 6 horses.

<table>
<thead>
<tr>
<th>Plasma drug concentration</th>
<th>Horse No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>AZA (ng/mL)</strong></td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>41.1</td>
</tr>
<tr>
<td>30 min</td>
<td>37.5</td>
</tr>
<tr>
<td>45 min</td>
<td>40.2</td>
</tr>
<tr>
<td>60 min</td>
<td>21.1</td>
</tr>
<tr>
<td>90 min</td>
<td>16.1</td>
</tr>
<tr>
<td>120 min</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td><strong>6-MP (ng/mL)</strong></td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td>30 min</td>
<td>23.0</td>
</tr>
<tr>
<td>45 min</td>
<td>30.0</td>
</tr>
<tr>
<td>60 min</td>
<td>13.4</td>
</tr>
<tr>
<td>90 min</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td>120 min</td>
<td>&lt; LOQ</td>
</tr>
</tbody>
</table>

*Time after AZA administration. LOQ = Limit of quantitation.*

Discussion

Results of our study indicate that in some respects, the pharmacokinetics of AZA and 6-MP in horses are similar to the pharmacokinetics of these agents in humans, although differences were found.²,²² For example, following IV administration, the elimination of AZA from the plasma in both species was rapid, but a substantial amount of variability between individuals was detected. In horses of our study, the elimination half-life of AZA was short with a mean ± SD value of 1.8 ± 0.4 minutes. In a similar manner, in a study in human renal transplant patients, the elimination half-life was short and was not significantly affected by renal function. For example, in patients with a creatinine clearance of < 25 ml/min, the elimination half-life was 9.3 ± 3.0 minutes, whereas in patients with a creatinine clearance > 25 ml/min, it was not significantly different at 12.5 ± 3.0 minutes.²² A longer mean elimination half-life of AZA of 50 minutes was found in a study of uremic human patients, but this was attributed more to the use of a sensitive analytic method for AZA determination than to the effect of renal failure on AZA elimination.²² Results of that study also found a large variability between individuals, with determinations of this parameter varying from 15 to 137 minutes.²² The AZA elimination half-life of horses in our study was similarly variable, ranging from 1.4 to 2.4 minutes.

Whereas the oral bioavailability of AZA in humans is generally poor, it was also poor in horses of our study. For example, following oral administration of AZA to uremic patients, the mean bioavailability was 18%.²² In contrast, in horses of our study, the mean ± SD bioavailability of AZA was 4 ± 3%. In humans, the low bioavailability of AZA has been proposed to result from a high first-pass effect, reflecting the rapid conversion of AZA to 6-MP and the uptake of 6-MP into lymphocytes and erythrocytes in the portal blood and liver.²² This theory is based to some extent on the clinical efficacy of orally administered AZA in humans, despite what appears to be a poor bioavailability. Also in support of this theory, results of 1 study found that...
when an $^{35}$S-labeled AZA compound was administered to human patients, 70% of the radiolabel was recovered in urine within 48 hours of AZA administration. It is impossible to determine from our study whether the poor bioavailability of AZA and 6-MP in our horses reflects rapid absorption and conversion of the compounds to active metabolites, as has been proposed to occur in humans, or simply poor absorption of the drug in this species.

Therapeutic drug monitoring of TPMT, AZA, 6-MP, and their active metabolites 6-TGNs in human patients is controversial. There appears to be a poor correlation between the pharmacokinetic parameters of AZA and 6-MP and their pharmacodynamic effects. This is particularly true when AZA is given orally because the low plasma concentrations that occur following this route of administration may nevertheless be associated with adequate immunosuppressive effects. Results of our study in horses are consistent with the findings in human patients. The elimination of AZA and 6-MP from plasma was rapid, and the bioavailability of the compounds was low. Therefore, trying to correlate plasma concentrations of AZA or 6-MP with clinical efficacy in horses would be difficult by use of this approach.

In human patients, results of several studies to correlate clinical efficacy and toxic effects of AZA and 6-MP with 6-TGN concentrations in erythrocytes or leukocytes are conflicting. For example, results of studies evaluating the efficacy of AZA in the treatment of inflammatory bowel disease in humans revealed a positive correlation between erythrocyte 6-TGN concentrations and therapeutic efficacy, whereas others failed to demonstrate such an association. In another study, 6-TGN concentrations in lymphocytes and neutrophils were significantly different from those in erythrocytes, which calls into question the value of monitoring erythrocyte concentrations. Whether monitoring of 6-TGN concentrations in equine erythrocytes or leukocytes could be correlated with clinical efficacy or toxicity in horses remains to be determined.

Despite the complex pathway of AZA and 6-MP metabolism in human patients, toxic effects of these compounds correlate well with the amount of TPMT present in erythrocytes. In humans, a single polymorphism controls TPMT activity. People homozygous for the TPMT high-activity allele compose 88.6% of the population and possess TPMT activity > 13.8 U/mL of RBCs. In contrast, people homozygous for the TPMT low-activity allele compose 0.3% of the population and possess TPMT activity of < 5 U/mL of RBCs. The remainder of the human population (11.1%) is heterozygous for the 2 alleles with TPMT activity between 5 and 13.8 U/mL of RBCs. Low TPMT activity in RBCs in humans and rodents correlates well with low TPMT activity in the liver and other organs. Clinically, low TPMT activity in humans has been associated with an increased risk of myelosuppression with AZA administration. Cats have also been reported to have low TPMT activity in RBCs, which has been hypothesized to be the cause of that species’ predisposition to develop myelosuppression when administered AZA. In contrast, dogs were reported to have high TPMT activity in RBCs ranging in 1 study from 15.1 to 26.6 U/mL. Consistent with this finding, AZA-associated myelosuppression in dogs, although recognized, is not common. Recent reports have expanded the range of TPMT activity in healthy dogs (7.9 to 71.8 U/mL of RBCs), but have not been able to demonstrate a correlation between TPMT activity in RBCs and myelosuppression. The range of TPMT activity in RBCs in horses of our study (1.5 to 2.6 U/mL of RBCs) was similar to those previously reported, and as such, horses would be considered to have low TPMT activity similar to cats and humans with the TPMT low-activity allele.

Despite the low TPMT activity in RBCs detected in horses of our study, no clinical or hematologic adverse effects occurred while receiving chronic AZA treatment. Several possible explanations exist for these results. First, systemic metabolism of the agent may be irrelevant when AZA is administered orally to horses as a result of the poor bioavailability (< 5%) of AZA in this species. Second, it is possible that a non–TPMT-dependent mechanism for the metabolism of the drug, such as xanthine oxidase, may be of greater importance than TPMT for the elimination of 6-MP in horses. Finally, TPMT activity in RBCs in this species may not correlate well with activity in other organs, such as the liver, where most of the AZA metabolism is believed to occur.

The cause of the nonpruritic alopecia in 1 horse in our study is unknown. It may have been caused by a Trichophyton infection secondary to immune suppression by the AZA, which subsequently resolved spontaneously once the AZA was discontinued. However, no histologic evidence of dermatophyte infection was found, only 1 colony of the dermatophyte was cultured, none of the in-contact horses developed alopecia, and dermatophytes can be found on the skin of normal horses. These reasons all make dermatophytosis as the cause of the alopecia less likely. It is interesting that this horse was also the only one to develop lymphopenia, although its development was transient.

In summary, when AZA was administered to horses IV, the AZA molecule and the active metabolite, 6-MP, were eliminated rapidly from the plasma. Following oral administration of the drug to horses, the bioavailability of AZA and 6-MP was poor, although whether this was the result of poor absorption or a high first-pass effect could not be determined in our study. Chronic oral administration of AZA was not associated with any clinically important adverse effects. Because of the safety of the AZA doses used in our study and the successful use of the drug in a small number of previous reports at or below the doses used in our study, further investigation into the clinical efficacy of AZA in the treatment of autoimmune diseases in horses is warranted.
References


