Pharmacokinetics, pharmacodynamics, and safety of zoledronic acid in horses

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Objective—To determine the pharmacokinetics, pharmacodynamics, and safety of zoledronic acid in horses.

Animals—8 healthy horses.

Procedures—A single dose of zoledronic acid (0.057 mg/kg, IV) was administered during a 30-minute period. Venous blood was collected at several time points. Zoledronic acid concentration in plasma was measured by liquid chromatography–tandem mass spectrometry, and pertinent pharmacokinetic parameters were determined. Plasma was analyzed for total calcium, BUN, and creatinine concentrations and a marker for bone resorption (C-terminal telopeptides of type I collagen).

Results—Zoledronic acid was safely administered IV during a 30-minute period, and no adverse effects were observed. Plasma concentrations of zoledronic acid were consistent with a 2-compartment mammillary model. Plasma concentrations of zoledronic acid were detected for up to 8 hours after administration. Mean total calcium concentrations in plasma were less than the reference range 7 days after zoledronic acid administration. A marker for bone remodeling decreased in concentration after zoledronic acid administration and remained low for the 1-year duration of the study. No changes in BUN and creatinine concentrations were observed after zoledronic acid administration.

Conclusions and Clinical Relevance—Zoledronic acid was safely administered in healthy horses. Zoledronic acid is reported as the strongest bisphosphonate presently available, and studies evaluating potential benefits of zoledronic acid in horses with orthopedic conditions are warranted. (Am J Vet Res 2013;74:550–556)

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Bisphosphonates are therapeutic agents with strong affinity for bone that markedly inhibit osteoclast activity. For that reason, bisphosphonates are often used in the treatment of skeletal diseases associated with high osteoclast activity and accelerated bone turnover (osteoporosis and Paget’s disease of bone). In addition, bisphosphonates are potent inhibitors of tumor-induced bone resorption and are commonly used in human patients with cancer (myeloma, breast cancer, and prostate cancer). On the basis of chemical structure and molecular mechanism of action, bisphosphonates are divided into 2 pharmacological classes (non–nitrogen-containing and nitrogen-containing bisphosphonates). The non–nitrogen-containing bisphosphonates are incorporated as cytotoxic ATP analogues in osteoclasts, triggering apoptosis by inhibiting mitochondrial ADP-ATP translocase. The nitrogen-containing bisphosphonates act by disrupting the function of small GTPases that are essential for osteoclast activity and survival.
Tiludronate is a non–nitrogen-containing bisphosphonate licensed in Europe for treatment of degenerative joint disease of the tarsus, signs of back pain, and navicular disease in horses. In human medicine, tiludronate is used for treatment of Paget’s disease of bone; however, stronger bisphosphonates are now routinely used for other conditions. The most potent bisphosphonates presently available for the treatment of osteoporosis or the management of neoplastic skeletal complications is zoledronic acid, also known as zoledronate. A recent study from the present authors’ laboratory used zoledronic acid in 10 horses with bone fragility disorder (silicate-associated osteoporosis) using an extrapolated dose from human medicine (0.057 mg/kg). Improvement of lameness score or resolution of the clinical signs was observed in 9 of 10 horses, and improvement on scintigraphic evaluation was ob-

ABBREVIATIONS

CTX-1 C-terminal telopeptides of type I collagen
TMS-DAM Trimethylsilyldiazomethane
served in 8 of 10 horses. In addition, a recent abstract described the use of zoledronic acid in 36 patients with orthopedic conditions, with no reports of long-term complications. However, potential species-specific pharmacological differences require that pharmacological agents be evaluated in the species of interest. Therefore, the objectives of the study reported here were to determine the pharmacokinetics, pharmacodynamics, and safety of zoledronic acid in horses.

Materials and Methods

Eight healthy horses from the Center for Equine Health, University of California-Davis, selected on the basis of results of history, physical examination, CBC, and serum biochemical analyses were included in the study. There were 4 geldings and 4 mares (median age, 15 years; range, 13 to 23 years; median weight, 525 kg; range, 480 to 590 kg). A physical examination was performed before, during, and immediately after zoledronic acid administration, then every 12 hours for 3 days and subsequently every time blood was collected. All procedures were approved by the University of California-Davis Institutional Animal Care and Use Committee.

Zoledronic acid administration and blood collection—Zoledronic acid was prepared immediately before administration according to the manufacturer’s instructions. Briefly, 100 mg of zoledronic acid was aseptically dissolved in 100 mL of a solution of sodium citrate (1 mg/mL), filtered with a 0.2-µm pore-size filter, and, immediately before administration, diluted (0.057 mg of zoledronic acid/kg) in 400 mL of sterile water and 100 mL of mannitol (20%) to a final concentration range of 0.034 µg/mL to 0.067 µg/mL, depending on each horse’s weight.

A 16-gauge jugular catheter was aseptically placed in each jugular vein for zoledronic acid administration and blood collection. Zoledronic acid was administered at 0.057 mg/kg during 30 minutes (20 mL/min) by means of one of the jugular catheters. Blood (10 mL) was collected from the other jugular catheter into 10-mL lithium heparin glass tubes immediately before zoledronic acid administration (baseline) and 1, 3, 5, 10, 15, 20, 30, 45, 60, and 90 minutes, and 2, 3, 4, and 8 hours after administration and by direct venipuncture at 24 hours, 4 days, 7 days, 28 days, 9 weeks, 6 months, and 1 year after administration. Immediately after collection, blood was centrifuged at 20°C for 3 minutes at 17,530 X g, and the plasma was collected and stored at −80°C until analysis.

Zoledronic acid determination—Zoledronic acid was quantitated in equine plasma by liquid chromatography–tandem mass spectrometry analysis following solid-phase extraction of plasma samples. The calibration standards were prepared as follows: stock solutions were made by dissolving 10.0 µg of zoledronic acid standard in 10.0 mL of methanol. Three zoledronic acid working standard solutions were prepared in methanol at 100, 10, and 1.0 µg/mL. Serial dilutions were prepared from the zoledronic acid standard solution by dilution of 10 µL of the 100, 10, or 1.0 µg/mL standard with methanol in a volumetric flask. Derivatized zoledronic acid and internal standards (etidronate acid) were prepared by the addition of TMS-DAM (2.0 mol/L solution in ethyl ether [50 µL]).

Plasma calibrators were prepared by dilution of the working zoledronic acid solutions with drug-free plasma to concentrations of 5, 10, 20, 50, 100, 250, and 500 ng/mL. Calibration curve and negative control samples were prepared fresh for each quantitative assay. The quality control samples (plasma with zoledronic acid at 2 concentrations [20 and 200 ng/mL]) were routinely included as an additional check of accuracy.

Plasma samples and calibrators were processed for analysis by diluting 500-µL aliquots with 2 mL of 0.01 formic acid (pH, approx 3.7), and internal standard solution (250 ng/mL) was added, followed by centrifugation (17,530 X g for 3 minutes). Solid-phase extraction was performed with columns (3 mL, 200 mg) conditioned sequentially with 3 mL of methanol and 2 mL of 0.01 formic acid (pH, approx 3.7). The plasma samples were loaded onto the column at a flow rate of 1 to 2 mL/min by use of low-pressure nitrogen gas (N₂). The columns were rinsed with 3 mL of water and 3 mL of methanol. Each column cartridge was dried by use of N₂ (20 psi) for 5 minutes. To collect the zoledronic acid, 3 mL of a 15% NH₄OH in methanol solution was applied to the column, and the fraction was collected. The eluent was dried in a nitrogen evaporator at 45° to 55°C for 20 to 30 minutes. The dried samples were reconstituted in a mobile phase mixture (160 µL).

Liquid chromatography–tandem mass spectrometry was performed on a mass spectrometer equipped with a heated electrospray ionization source and interfaced with an autosampler. The most abundant ion transition (ie, m/z 329.1 to 203.1) for zoledronic acid–TMS-DAM acid was used for quantification. The second (ie, m/z 329.1 to 171.0) and third (ie, m/z 329.1 to 134.9) most abundant transitions were used as qualifier transitions. All standards, controls, calibrators, and samples were prepared in duplicate and peak ion area ratios of the analyte and internal standard TMS-DAM (m/z 331.1 to 206.1) were calculated. The response for the product ions for zoledronic acid–TMS-DAM (m/z 203.1) were plotted and peaks at the proper retention time integrated by use of quantitative software. The software was used to generate calibration curves and quantitate these analytes in all samples. The limits of detection and quantification of the assay were 1 and 5 ng/mL, respectively.

Quality control and sample acceptance criteria were specified according to the guidelines and standard operating procedures of the Kenneth L. Maddy Laboratory. The requirement was that the coefficient of variation for all calibrators, positive controls, and samples must not exceed 10% (15% at the lower limit of quantitation).

Total calcium, creatinine, BUN, and bone biomarker evaluations—Total calcium in plasma of the horses was measured before zoledronic acid infusion and 8 hours, 24 hours, 4 days, 7 days, 28 days, and 9 weeks after administration. Creatinine and BUN concentrations were measured in plasma before zoledronic acid administration and 7 days after administration.

The specific biomarker for bone resorption,CTX-1, was measured in plasma at baseline, 7 days,
Samples were analyzed simultaneously in duplicates; products of CTX-1 in human serum and plasma. Immunologic test for the quantification of degradation products of CTX-1 in human serum and plasma.

Pharmacokinetic analysis—Data were analyzed with a commercial pharmacokinetic data analysis software package. Data were modeled via both noncompartmental and compartmental analysis. A 2-compartmental model was found to be the best fit on the basis of coefficient of variation, Akaike information criterion, and visual examination of the line fit and residual plots. Weighting of the data using the inverse square of the concentration improved the line fit and residual plots and was used for all data.

Statistical analysis—Total calcium and bone biomarker concentrations after zoledronic acid administration were compared with preinfusion values by use of a repeated-measures ANOVA with a Dunnett post hoc analysis. Creatinine and BUN concentrations were compared before and after zoledronic acid infusion by use of a paired t test. Analysis was performed with commercial software. A value of $P < 0.05$ was considered significant.

Results

Zoledronic acid was safely administered over 30 minutes, and no adverse effects were observed. Concentrations of zoledronic acid decreased rapidly after administration, and by 8 hours, all horses had no detectable plasma concentrations. The pertinent pharmacokinetic parameters for the noncompartmental and 2-compartmental analysis were determined (Table 1). Data from 1 horse did not fit the 2-compartmental model and were removed from the 2-compartmental results.

All horses had baseline total calcium concentrations within the reference range from our laboratory (Figure 2). On day 7, mean total calcium concentration was less than the reference range (11.4 to 14.1 mg/dL), but was not significantly different from baseline value. Concentrations of creatinine and BUN were within reference range for all horses on day 7 and were not significantly different from baseline. A significant decrease from baseline values in CTX-1 concentration was detected at all times evaluated after treatment (Figure 3).

Discussion

All bisphosphonates currently in use today have pyrophosphate-like substructures with a carbon atom joining the 2 phosphates (P-C-P). Nitrogen-containing bisphosphonates have a hydroxyl group on the carbon atom that is poorly metabolized and confers high affinity for bone surfaces. Opposite to the hydroxyl radical, the carbon atom contains a nitrogen-containing side chain responsible for its potency. Zoledronic acid is a member of the nitrogen-containing bisphosphonates and acts on bone to decrease osteoclast-mediated absorption was measured via an automated ELISA plate reader, and concentrations were calculated via a quadratic curve fit.

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bone resorption. Of all bisphosphonates, zoledronic acid has the strongest affinity for hydroxyapatite, with preferential localization at sites of high bone turnover. Early bisphosphonates do not have the nitrogen-containing side chain and most (clodronate and tiludronate but not etidronate) also do not have the hydroxyl group, causing them to lack sufficient potency and bone specificity to be efficacious and safe. In addition, nitrogen-containing bisphosphonates have a different mechanism of action than bisphosphonates that do not contain nitrogen. Zoledronic acid is used in humans for the treatment of osteoporosis, Paget’s disease of bone, and skeletal metastases. The recommended dose and frequency of administration of zoledronic acid in human patients vary depending on the condition being treated. For the treatment of osteoporosis, an infusion of 5 mg every 1 or 2 years is recommended. In Paget’s disease of bone, a single dose of 5 mg is effective to control the disease for at least 5 years. For cancer patients with hypercalcemia of malignancy, an infusion of 4 mg is recommended and can be repeated after 7 days, and in patients with multiple myeloma or bone metastasis from solid tumors, the recommended dose is 4 mg every 3 to 4 weeks. For the present study the authors chose a dose similar to the 4-mg dose used in humans, assuming a 70-kg patient (0.057 mg/kg). In humans, zoledronic acid is administered during a period of no less than 15 minutes to prevent adverse effects. We decided to administer the zoledronic acid at a slower infusion rate to reduce even more the possibility of adverse effects.

Pharmacokinetic studies of zoledronic acid have been performed in healthy dogs and humans with cancer. No pharmacokinetic data are available for zoledronic acid in humans with osteoporosis or healthy individuals. Most of the pharmacokinetic studies in humans used a dose (4 mg) similar to the one used in this study. However, the study in healthy dogs used a single dose 4.4 times our selected dose. Because of renal adverse effects observed in humans with higher doses of zoledronic acid, it is recommended not to exceed the 4- to 5-mg dose for adults. Studies in humans with cancer reveal that administration of zoledronic acid results in dose-proportional plasma concentrations that decrease in a multiphasic manner after IV administration. The plasma concentration of the drug has a rapid triphasic decrease, with values at 4 and 24 hours of < 10% and 1% from concentrations at the end of the infusion, respectively. Similarly, in the present study, a rapid decrease of zoledronic acid concentrations was observed, with only 6% of the postinfusion concentration present by 3 hours after infusion. A study in humans revealed a multiphase decrease in plasma concentrations, considered as a 3- or 4-compartment mammillary model. The concentrations observed in the present study after administration of zoledronic acid were consistent with a 2-compartment mammillary model and were similar to those observed in the study using healthy dogs. The more complex pharmacokinetic model observed in human studies, compared with that in dogs and the present study, may be explained, at least in part, by the detection of measurable plasma concentrations for longer periods. By 4 hours, only 2 of the 8 horses had measurable plasma concentrations of zoledronic acid, and by 8 hours, all horses had undetectable concentrations. It seems likely that...
additional phases of elimination would have been observed if a more sensitive assay had been used. Bisphosphonates do not have the strong chromophores typically used for UV detection in high-performance liquid chromatography methods. Neither separation using liquid or gas chromatography nor detection by mass spectrometry has sufficient analytic reproducibility and sensitivity. In addition, the functional moiety of zoledronic acid is highly negatively charged and binds strongly to calcium crystals to form insoluble calcium salts. To improve sensitivity, a radioimmunoassay using polyclonal antibodies against zoledronic acid and an iodine 125 zoledronic acid derivative used as a tracer was created. The radioimmunoassay has a limit of quantitation less than one-tenth that of the assay used in the present study; however, it is not commercially available. In addition, zoledronic acid is rapidly removed from the circulation, with plasma concentrations <1% of maximum concentration by 24 hours in a human study. Within 24 hours of administration, 41% of the dose infused is eliminated in the urine of humans, suggesting that 60% is retained in the skeleton. It has been proposed that the long duration of action is due to a continuous recycling of bisphosphonates off and back onto the bone surfaces. Bisphosphonates have a high affinity for hydroxyapatite crystals of bone, where they can be stored for years. During bone resorption, the acidic pH of the subcellular space causes dissolution of bone mineral with the release of bisphosphonates that are then internalized by osteoclasts, interfering with specific biochemical processes. Because zoledronic acid is retained in the bones for several years, whereas circulating concentrations are detected for only days, the pharmacokinetics of the drug may not predict its pharmacoodynamic effects.

Peak plasma concentration of zoledronic acid in human patients decreases when the infusion time is increased. The mean plasma concentration of zoledronic acid in the horses in the present study (54 µg/L) was considerably less than that reported in humans (264 µg/L). However, infusion rate was twice as long as in the human study, and this may be responsible for the differences between studies. A dose based on exact weight was used in the present study, compared with the fixed dose used in humans. The mean weight of humans varies depending on country of residence and sex and is lower in osteoporotic patients and those with cancer; therefore, the doses used in the humans may be higher on a milligram-per-kilogram basis. In addition, differences in zoledronic acid determination and species variability may also be partially responsible. The clearance of 0.9 L/h/kg determined in the present study was approximately 4 and 10 times that observed in dogs and human patients with cancer when normalized for body weight.

The most common adverse effects after administration of zoledronic acid are renal toxicity and acute-phase reactions; less common adverse effects include osteonecrosis of the mandible, hypocalcemia, ocular complications, erythema, phlebitis, and adverse effects on the CNS. Zoledronic acid has been associated with acute tubular necrosis that has progressed to acute fatal renal failure in some cases. However, renal problems are more commonly observed at high doses and with reduced infusion times. Because of the renal safety issues associated with zoledronic acid, the manufacturer advises to evaluate blood creatinine concentration before administration. We did not observe renal problems after zoledronic administration in any horses. All horses were clinically healthy and were not receiving NSAIDs. Blood creatinine and BUN concentrations were evaluated before and after zoledronic acid administration and were within reference range. In an attempt to decrease the risk of potential adverse renal effects, the infusion time was extended from 15 to 30 minutes. Acute-phase reactions are reported after IV administration of bisphosphonates in humans. Clinical signs include flu-like symptoms, particularly low fever, leukocytosis, exhaustion, and muscle and joint pain. Symptoms generally resolve within 48 hours and respond well to NSAIDs. The cause of acute-phase reactions is a transient increase in inflammatory cytokines (interleukin-6 and tumor necrosis factor). Pyrexia has been reported after zoledronic acid administration in patients with cancer (26% to 36%) and osteoporosis (9%); however, placebo-treated patients have similar prevalence. Fever or colic was not observed in any of the horses in the present study. It is possible that subtle signs were present, but the horses were monitored in a hospital setting after administration. In the clinical cases treated at the authors’ hospital with zoledronic acid, the horses were premedicated with flunixin meglumine (IV, 1 mg/kg) 30 minutes before infusion to minimize the possibility of acute-phase reactions.

Over recent years, cases of osteonecrosis of the jaw have emerged in patients receiving bisphosphonates. However, osteonecrosis of the jaw after zoledronic acid administration is often observed in patients with cancer or after dental procedures rather than in people with osteoporosis. Dental problems were not observed in any of the horses in the present study for as long as a year after treatment. Because most cases are reported after dental procedures, we recommend that no dental procedures be performed immediately before zoledronic acid administration or for 6 months afterwards. In a large-scale study using zoledronic acid for the treatment of postmenopausal osteoporosis, hypocalcemia was reported in 1.27% of patients, from 9 to 10 days following the infusion. In the present study, a mean total calcium value less than reference range was detected at day 7 after administration. Although clinical signs of hypocalcemia were not observed in this study, the authors have observed severe hypocalcemia (total calcium, 4.8 mg/dL [reference range, 11.4 to 14.1 mg/dL]; ionized calcium, 0.6 mmol/L [reference range, >1.3 mmol/L]) in a horse 11 days after zoledronic acid infusion. Therefore, we recommend measuring calcium concentration prior to administration and use of either a diet rich in calcium (alfalfa) or oral calcium supplementation from a week before infusion until at least 3 weeks after treatment. The time at which plasma calcium concentration will be lowest after zoledronic acid administration is unknown for horses. Knowing that in 1 clinical case severe hypocalcemia developed 11 days after infusion, it would have been useful in our research horses to add more time points between days 7...
and 28. However, the authors have observed a substantial reduction in total blood calcium concentrations in horses treated with zoledronic acid for a bone fragility disorder on day 7 and a mild decrease on day 21. In the present study, horses had mean total blood calcium concentration on week 9 (11.2 mg/dL) slightly lower than reference range; the importance of this finding is unknown. In humans, symptoms of hypocalcemia resolve with initial calcium replacement even though serum calcium concentration may still be less than reference range. In addition, serum calcium has taken up to 60 days to return to reference range in a case report. Studies evaluating safety of other bisphosphonates in horses are limited. In 5 horses, tiludronate administration at 1 mg/kg, IV, induced a transient increase in heart rate at 30 minutes and 2 hours and transient hypocalcemia at 30 minutes. The informational insert of the commercial tiludronate formulation describes signs of colic, restlessness, recumbency, softening of feces, and sweating in <11% of horses treated.

Pharmacodynamic effects of bisphosphonates are commonly estimated by means of osteoporotic fracture risk reduction, bone mineral density, imaging techniques, and measurement of biomarkers of bone metabolism in plasma or urine. Blood biomarkers of bone metabolism have been successfully tested in horses, including CTX-1 as a marker of bone resorption and bone-specific alkaline phosphatase as a marker of bone formation. Studies in postmenopausal women have observed a decrease in plasma CTX-1 concentration and bone-specific alkaline phosphatase activity for up to a year after administration. Similarly the present study detected decreased CTX-1 concentration from baseline until 1 year after infusion (last samples collected). Plasma CTX-1 concentration is considered one of the most relevant markers in the monitoring of a bisphosphonate treatment and reflects the general bone resorption state of an individual. Administration of tiludronate in healthy horses induced a significant decrease in CTX-1 concentration for only 3 days after infusion. Zoledronic acid is considered the most potent available bisphosphonate and has been found to have 200 to 2,000 times the potency of non-nitrogen-containing bisphosphonates. Some bisphosphonates (alendronate and zoledronic acid) have long lasting effects in reducing bone turnover, compared with others (etidronate and risedronate). These differences in retention in the body and persistence of effect are suggested to be related to differences of affinity for hydroxyapatite. By comparing the duration of the reduction in plasma CTX-1 concentration, it appears that zoledronate induced a longer effect on bone resorption than tiludronate, in horses. It would have been desirable to collect samples for an extended period to determine the duration of the effect of zoledronic acid in markers of bone remodeling in horses.

Results of the present study suggested that zoledronic acid can be safely used in horses as a continuous infusion over 30 minutes of a similar dose (0.57 mg/kg) to that recommended for humans patients (4 mg). Because of the reported adverse effects of zoledronic acid, it is recommended to evaluate kidney function and calcium concentration before administration, provide calcium supplementation before and after treatment, and limit dental work before and after administration. Zoledronic acid is reported as the strongest bisphosphonate currently available, and equine studies evaluating potential benefits of zoledronic acid in clinical cases are warranted.

References
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