Pharmacokinetics and tissue concentrations of azithromycin in ball pythons (*Python regius*)

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Objective—To determine pharmacokinetics and tissue concentrations of azithromycin in ball pythons (*Python regius*) after IV or oral administration of a single dose.

Animals—2 male and 5 female ball pythons.

Procedures—Using a crossover design, each snake was given a single dose of azithromycin (10 mg/kg) IV. After a 4-week washout period, each snake was given a single dose of azithromycin (10 mg/kg) orally. Blood samples were collected prior to dose administration and 1, 3, 6, 12, 24, 48, 72, and 96 hours after azithromycin administration. Azithromycin was quantitated by use of liquid chromatography-mass spectrometry.

Results—After IV administration, azithromycin had an apparent volume of distribution of 5.69 L/kg and a plasma clearance of 0.19 L/h/kg. Harmonic means for the terminal half-life were 17 hours following IV administration and 51 hours following oral administration. Mean residence times were 37 and 94 hours following IV and oral administration, respectively. Following oral administration, azithromycin had a peak plasma concentration (C_{max}) of 1.04 µg/mL, a time to C_{max} of 8.4 hours, and a prolonged mean absorption time of 57 hours. Mean oral bioavailability was 77%. Tissue concentrations ranged from 4 to 140 times the corresponding plasma concentration at 24 and 72 hours after azithromycin administration.

Conclusions and Clinical Relevance—Azithromycin is well absorbed and tolerated by ball pythons. On the basis of plasma pharmacokinetics and tissue concentration data, we suggest an azithromycin dosage in ball pythons of 10 mg/kg, orally, every 2 to 7 days, depending upon the site of infection and susceptibil ity of the infective organism. (*Am J Vet Res* 2003;64:225–228)

Azithromycin is a member of a subclass of macrolide antimicrobials classified as azalides. The chemical structure of azithromycin is similar to erythromycin but with the addition of a methyl-substituted nitrogen in the lactone ring creating a 15-membered azalide.[1] The change in structure improves acid stability and tissue penetration, compared with erythromycin. The mechanism of action is also similar to the inhibition of protein synthesis by binding of the 50S ribosomal subunit.[2,3]

Azithromycin provides broad-spectrum antibiosis with some activity against anaerobic organisms.[4] Results of recent studies indicate that azithromycin has activity against *Mycoplasma*, *Chlamydia*, *Toxoplasma*, *Borrelia*, *Cryptosporidium*, *Giardia*, and *Plasmodium* spp, and the *Mycobacterium avium* complex.[5-12]

Azithromycin administration results in sustained drug concentrations in tissues that are greater than the corresponding plasma concentration. The drug rapidly moves from plasma into the intracellular compartments, especially in the pulmonary, lymphatic, and genital tissues.[13,14] At equilibrium in humans, azithromycin concentrations in tissues are up to 200× greater than in plasma. Azithromycin also accumulates within WBCs, which allows azithromycin to be carried directly to the site of infection.[15,16]

Stability and tissue penetration characteristics of azithromycin are desirable in the treatment of bacterial diseases of reptiles. The ball python (*Python regius*) is a species that is representative of the Boidae family. Therefore, the purpose of the study presented here was to determine the pharmacokinetics and tissue concentrations of azithromycin in ball pythons after IV or oral administration of a single dose. Data derived from our study will be used in designing therapeutic dosage regimens for treating infectious bacterial diseases of ball pythons.

Materials and Methods

Animals—The Institutional Animal Care and Use Committee of Kansas State University approved our study. Seven ball pythons (2 males, 5 females) weighing 0.67 to 0.96 kg were used. Each snake was housed individually in a 114-L aquaria with newspaper substrates and screen tops. Snakes had access to a hide box and water ad libitum. Cages were housed in a thermostatically controlled room at 30°C with a 12-hour light-dark cycle.[17] A physical examination and CBC determination were performed for each snake prior to the start of our study.

Study design—In the first phase of our study, a crossover design was used to evaluate the pharmacokinetics of azithromycin. Each snake was given a single dose of azithromycin[a] (10 mg/kg) via cardiocentesis. After a 4-week washout period, each snake was given a single dose of azithromycin (10 mg/kg) orally by use of the same preparation. Blood samples (0.75 mL) were collected prior to dose administration (at least 1 week) and at 1, 3, 6, 12, 24, 48, 72,

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and 96 hours after azithromycin administration. Samples were immediately transferred to evacuated lithium heparin tubes.[b] Plasma was separated via centrifugation (10 minutes, approx 2000 × g) and stored at –70°C until analyzed.

In the second phase of our study, 6 snakes were used to determine tissue concentrations of azithromycin. Snakes had not received a dose of azithromycin for a period of 4 months. Each snake was given a single dose of azithromycin (10 mg/kg) orally. Three snakes were euthanatized 24 hours after dose administration, and the remaining 3 snakes were euthanatized 72 hours after dose administration. Blood samples and liver, kidney, lung, and skin specimens were collected and stored at –70°C until analyzed. For euthanasia, tiletamine-zolazepam[c] (25 mg/kg, IM) was administered first to anesthetize the snakes. After a cut down to the vena cava and blood collection from this vein, pentobarbital and phenytoin solution[d] (1 mL/4.5 kg) were administered via the vena cava to euthanatize each snake.

Plasma and tissue analysis for azithromycin—Blood samples and tissue specimens were analyzed via liquid chromatography-mass spectrometry by use of previously described methods for extraction of the samples[13] and chromatographic conditions.[18] Accuracy and precision were within ± 15% of actual values, and recovery was > 80% across the range of the assay in all fluids and tissues. The plasma standard curve had a linear range of 0.015 to 2.00 µg/mL. The limit of quantitation was 0.013 µg/mL for plasma. The tissue standard curve had a linear range of 0.25 to 15 µg/g with a limit of detection of 0.22 µg/g.

Pharmacokinetic calculations—Values of pharmacokinetic parameters were determined for each snake by use of noncompartmental analysis[19,20] with a commercial software program.[c] Values calculated following the IV administration of azithromycin were as follows: plasma **area under the concentrations versus time curve (AUC)**; **area under the first moment curve (AUMC)**; **mean residence time (MRT)**, where MRT = AUMC/AUC; **apparent volume of distribution at steady state (V_d)**, where V_d = (dose × AUMC/AUC^2); **plasma clearance (Cl_p)**, where Cl_p = dose/AUC; **elimination rate constant (k_{el})** calculated as the slope of the terminal phase of the plasma concentration curve that included a minimum of 3 time points; and terminal **half-life ($t_{½}$)**, where $t_{½}$ = 0.693/k_{el}. Following oral administration of azithromycin, the following parameters were determined: AUC; AUMC; MRT; **mean absorption time (MAT)**, where MAT = MRT_{PO} – MRT_{IV}; and **bioavailability (F)**, where F = (AUC_{PO}/AUC_{IV}) × 100. The AUC and AUMC were calculated by use of the trapezoidal rule with extrapolation to infinity.

Results

All snakes were clinically normal upon physical examination prior to the start of our study. The CBC results were within reference range limits for our institution. Two weeks after IV administration of azithromycin, 1 snake died from apparent nonregenerative anemia. All other snakes remained clinically normal during and after both phases of our study.

In the first phase of our study, plasma AUCs (**Fig 1**) and pharmacokinetic parameters (**Table 1**) following IV and oral administration of azithromycin were determined. In the second phase of our study, concentrations of azithromycin in plasma samples and liver, kidney, lung, and skin tissues were determined following oral administration (**Fig 2**). Tissue-to-plasma concentration ratios for azithromycin were determined for each snake (**Table 2**). Because of the limited num-

Table 1—Azithromycin pharmacokinetic parameters in ball pythons following IV and oral administration of a single dose (10 mg/kg)

	Values				
Parameters	**Mean**	**SD**	**Median**	**Minimum**	**Maximum**
IV administration (n = 7)					
$AUC_{0-\infty}$ (h × µg/mL)	70	37	72	25	119
$AUMC_{0-\infty}$ (h^2 × µg/mL)	2909	2563	2441	130	8211
MRT (h)	37	19	35	5.2	69
V_d (L/kg)	5.69	3.16	4.70	2.08	10.1
Cl_p (L/h/kg)	0.190	0.114	0.139	0.0837	0.401
k_{el} (h^{-1})	0.041	0.061	0.0183	0.0081	0.180
$t_{½}$ (h)	17	HM	38	3.9	86
Oral administration (n = 6)					
$AUC_{0-\infty}$ (h × µg/mL)	45	22	43	20	82
$AUMC_{0-\infty}$ (h^2 × µg/mL)	4908	5187	2360	1688	14851
MRT (h)	94	50	76	53	181
MAT (h)	57	43	52	17	112
C_{max} (µg/mL)	1.04	0.382	0.948	0.538	1.50
T_{max} (h)	8.4	8.8	6.0	1.0	24
k_{el} (h^{-1})	0.014	0.0063	0.014	0.0059	0.022
$t_{½}$ (h)	51	HM	49	32	117
F (%)	77	27	69	49	128

$AUC_{0-\infty}$ = Area under the plasma concentration versus time curve from time of administration to infinity. $AUMC_{0-\infty}$ = Area under the first moment curve from time of administration to infinity. MRT = Mean residence time. V_d = Apparent volume of distribution at steady state. Cl_p = Plasma clearance. k_{el} = Elimination rate constant. $t_{½}$ = Half-life. HM = Harmonic mean. MAT = Mean absorption time. C_{max} = Peak plasma concentration. T_{max} = Time of peak plasma concentration. F = Bioavailability.

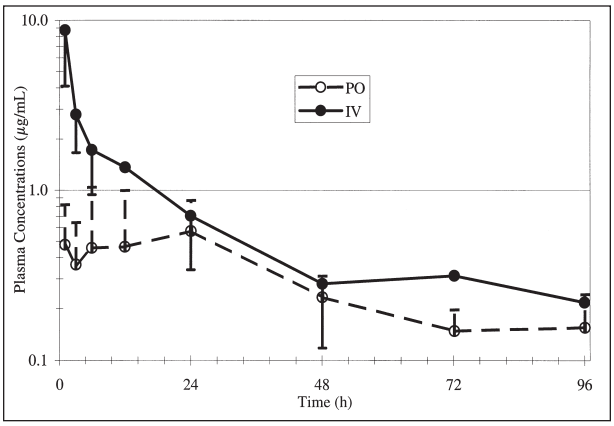

Figure 1—Mean (± SD) plasma concentrations (µg/mL) of azithromycin in ball pythons following IV and oral (PO) administration of a single dose (10 mg/kg).

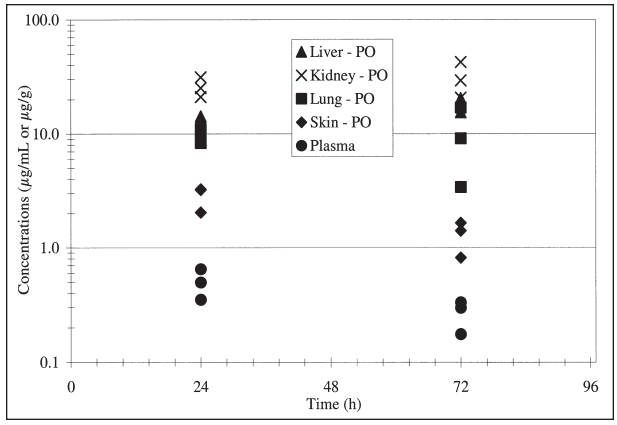

Figure 2—Individual plasma and tissue concentrations (µg/mL or µg/g, respectively) of azithromycin in ball pythons following oral administration of a single dose (10 mg/kg).

Table 2—Individual azithromycin tissue-to-plasma concentration ratios in ball pythons following oral administration of a single dose (10 mg/kg)

Tissue	24 h	72 h
Lung	13, 22, 29	19, 31, 51
Skin	4.1, 5.0, 9.2	4.6, 4.7, 5.0
Liver	22, 28, 34	52, 62, 102
Kidney	43, 48, 72	88, 118, 143

ber of time points for tissue concentrations, $t_{1/2}$ values were not determined.

Discussion

In human medicine, azithromycin is approved for use in treating respiratory tract, skin, and sexually transmitted diseases.[14,21] It has a long terminal plasma $t_{1/2}$ (70 hours) in humans.[22] Azithromycin is excreted approximately 75% unchanged in the bile of mammals, which indicates that the primary xenobiotic component is the unaltered drug compound.[1,13] Compared with other species, the $t_{1/2}$ for a single dose of orally administered azithromycin in the ball python is 51 hours, which is comparable to humans. The V_d determined in the snakes of our study (5.69 L/kg) is not large, compared with the V_d of rats (84 L/kg), cats (23 L/kg), and dogs (12 L/kg).[1,13,23] However, azithromycin in pythons appears to be distributed to a greater extent than amikacin (0.41 L/kg) or piperacillin (2.6 L/kg).[24,25] The larger V_d of azithromycin in the ball python supports the high concentrations of azithromycin found in tissue specimens (Fig 2) and the potential for greater efficacy against susceptible organisms, compared with other antimicrobials evaluated in pythons to date. It is unknown why the V_d of azithromycin in ball pythons is so much smaller in this species of reptile, compared with the V_d of azithromycin in the 3 mammalian species mentioned. The smaller V_d in ball pythons could be a result of a high rate of metabolism of azithromycin or the result of species differences in protein binding. Finally, the smaller V_d reported for ball pythons in our study could be the result of reptilian cellular anatomy, which may decrease the penetration of azithromycin into some or all tissues.

The mean bioavailability of azithromycin was 77% following oral administration of a single dose. This value is greater than the mean bioavailability of azithromycin in humans (37%) or cats (58%), but less than that in dogs (97%).[1,13,22] The variation in bioavailability of azithromycin in the snakes of our study may be the result of the prolonged absorption indicated by the values of MAT and time to reach maximal concentration. Also, feeding may affect intestinal absorption. When a snake consumes a meal, the small intestinal mucosa will increase in thickness by 2 to 3 times the prefeeding thickness, but total length of the small intestine does not change. Also, the villi length increases to 2 times greater than the prefeeding length. An increase in the surface area of the small intestine in response to a recent meal would allow for an increase in absorption of azithromycin. The intestine of snakes will regress in size during prolonged periods of not eating.[26-28] This factor may greatly enhance or restrict intestinal absorption of azithromycin or any pharmacologic agent in the gastrointestinal tract, depending upon the period between drug administration and the last feeding.

As with intestinal changes, metabolism is also affected by feeding.[27,28] Metabolism will peak about 1 week after feeding; however, there is minor metabolic investment in digestion after a period of not eating and energy reserve depletion. With variable states of metabolism, drug metabolism and elimination could be directly affected by feeding intervals. Snakes of our study received a dose of azithromycin 6 days after feeding in an attempt to achieve consistent rates of metabolism and elimination. The affect of feeding status will widely affect the way orally administered pharmacologic agents are absorbed by snakes.

Because of the accumulation of azithromycin in tissues, it is not evaluated on the basis of plasma concentrations alone. Data from our study indicate that concentrations of azithromycin are higher in tissues than in plasma (Fig 2). Even at 72 hours following administration, the concentration of azithromycin in the lung, liver, skin, and renal tissues is greater than the corresponding plasma values. *Aeromonas hydrophila* is 1 of the most common reptile pathogens, especially in infections of the respiratory tract and in cases of stomatitis.[29] The **minimum inhibitory concentration** that will result in death of 90% of the organisms (**MIC_{90}**) of azithromycin for *A hydrophila* is 4 µg/mL.[30] In our study, the concentration of azithromycin in skin specimens was slightly less than the reported MIC_{90} for *A hydrophila* at 24 hours after administration and much lower at 72 hours after administration. However, at 24 and 72 hours after administration, concentrations of azithromycin in lung specimens were 2 to 4 times greater than the reported MIC_{90} for *A hydrophilia*. Although azithromycin has been reported to be active against *Pseudomonas* infections in humans,[31] more research is need to determine the efficacy of azithromycin against this pathogen in snakes.

Compared with other antimicrobials, azithromycin (10 mg/kg, PO, q 2 to 7 d) may be effective for the treatment of susceptible microbes in reptiles because of its broad spectrum of activity, increased tissue penetration, and prolonged residence in tissues. The dose administration interval should be optimized on the basis of the MIC of azithromycin for the target organism and the location of the infection (eg, skin, q 3 d; respiratory tract, q 5 d; liver and kidney, q 7 d).

[a]Zithromax, Pfizer Inc, New York, NY.
[b]Vacutainer, Becton Dickinson & Co, Franklin Lakes, NJ.
[c]Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa.
[d]Beuthanasia-D Special, Schering-Plough Animal Health, Kenilworth, NJ.
[e]WinNonlin, version 3.1, Pharsight, Mountain View, Calif.

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