Pharmacokinetics of azithromycin and concentration in body fluids and bronchoalveolar cells in foals

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Objective—To determine the pharmacokinetics of azithromycin and its concentration in body fluids and bronchoalveolar lavage cells in foals.

Animals—6 healthy 6- to 10-week-old foals.

Procedure—Azithromycin (10 mg/kg of body weight) was administered to each foal via IV and intragastric (IG) routes in a crossover design. After the first IG dose, 4 additional IG doses were administered at 24-hour intervals. A microbiologic assay was used to measure azithromycin concentrations in serum, peritoneal fluid, synovial fluid, pulmonary epithelial lining fluid (PELF), and bronchoalveolar (BAL) cells.

Results—Azithromycin elimination half-life was 20.3 hours, body clearance was 10.4 ml/min·kg, and apparent volume of distribution at steady state was 18.6 L/kg. After IG administration, time to peak serum concentration was 1.8 hours and bioavailability was 56%. After repeated IG administration, peak serum concentration was 0.63 ± 0.10 µg/ml. Peritoneal and synovial fluid concentrations were similar to serum concentrations. Bronchoalveolar cell and PELF concentrations were 15- to 170-fold and 1- to 16-fold higher than concurrent serum concentrations, respectively. No adverse reactions were detected after repeated IG administration.

Conclusions and Clinical Relevance—On the basis of pharmacokinetic values, minimum inhibitory concentrations of *Rhodococcus equi* isolates, and drug concentrations in PELF and bronchoalveolar cells, a single daily oral dose of 10 mg/kg may be appropriate for treatment of *R equi* infections in foals. Persistence of high azithromycin concentrations in PELF and bronchoalveolar cells 48 hours after discontinuation of administration suggests that after 5 daily doses, oral administration at 48-hour intervals may be adequate. (*Am J Vet Res* 2001;62:1870–1875)

Azithromycin is an azalide antimicrobial commonly used in human medicine.[1] Azalides are similar to macrolides in that both inhibit bacterial protein synthesis by binding to subunits of the 50S ribosome. Azithromycin differs from macrolides in its structural composition by having a 15-member macrocyclic lactone ring containing a methylated nitrogen atom.[2] This

difference in composition gives azithromycin several advantages over erythromycin and other traditional macrolides. Compared with erythromycin, azithromycin has a higher oral bioavailability and a larger apparent volume of distribution as well as improved tissue and phagocytic cell uptake.[2-5] Azithromycin has a longer elimination half-life and prolonged tissue concentrations, compared with other antimicrobial agents, which allows once daily dosing and shorter duration of treatment.[2,4,5] Like erythromycin, azithromycin is effective against most gram-positive aerobes.[6,7] However, azithromycin is more active than erythromycin against gram-negative bacteria and anaerobes.[6,7] In humans, the incidence and severity of adverse reactions for azithromycin are also considerably decreased, compared with erythromycin.[1]

Erythromycin is commonly used in equine medicine for treatment of *Rhodococcus equi* infections in foals. *Rhodococcus equi,* a gram-positive facultative intracellular pathogen of macrophages, is the most devastating cause of pneumonia in foals between 3 weeks and 5 months of age. Although combined treatment with erythromycin and rifampin has dramatically improved the survival rate of foals infected with *R equi*, this treatment regimen is not without problems. Erythromycin has variable absorption in foals when given orally, requires multiple daily dosing, and, most importantly, has a high incidence of potentially fatal adverse effects.[8-10] Furthermore, in a recent study,[11] 13% of *R equi* isolates were found to be resistant to erythromycin. Progressive development of resistance during treatment has also been described.[12] Treatment of foals infected with erythromycin-resistant isolates is problematic because of the lack of effective alternatives. Therefore, there is a tremendous need for other effective and potentially safer antimicrobial agents to combat infection by this pathogen. Because of its numerous advantages over erythromycin, azithromycin is an attractive alternative for the treatment of *R equi* infections. Other potential indications for the use of azithromycin in foals include treatment of pneumonia or abscesses caused by *Streptococcus* spp as well as intracellular pathogens such as *Salmonella* spp. However, the lack of pharmacokinetic studies precludes the rational use of this antimicrobial agent in foals.

The purpose of the study reported here was to determine the pharmacokinetics of azithromycin in foals and its bioavailability and concentrations in serum, body fluids, and bronchoalveolar cells after repeated **intragastric (IG)** administration.

Materials and Methods

Horses and experimental design—Three female and 3 male foals (Thoroughbred [n = 3], Quarter Horse [2], and Quarter Horse-Thoroughbred cross [1]) between 6 and 10 weeks of age and weighing between 91 and 155 kg were selected for this study. The foals were considered healthy on the basis of history, physical examination, CBC, measurement of fibrinogen concentration, and plasma biochemical profile. The foals were kept with their dams at all times. They were kept at pasture between experiments and in individual stalls during the experiments, with ad libitum access to grass hay and water.

Azithromycin was administered at a dose of 10 mg/kg of body weight via the IV and the IG routes, using a crossover design. A washout period of at least 12 days was allowed between studies for the 2 dosing routes. For the IV study, injectable azithromycin[a] (100 mg/ml) diluted to a final volume of 60 ml in saline (0.9% NaCl) solution was administered as a bolus through a catheter placed in the left jugular vein. Blood samples were obtained from a catheter placed in the right jugular vein at 0 (prior to administration), 3, 6, 15, 30, 60, and 90 minutes and 2, 3, 4, 6, 8, 12, 24, and 48 hours after administration.

For the IG dosing route study, azithromycin tablets[b] (250 mg tablets) were dissolved in approximately 100 ml of water and administered by nasogastric tube. For the first 24 hours, blood samples were collected as described for the IV study. Afterwards, 4 additional IG doses were administered at 24-hour intervals (24, 48, 72, and 96 hours after the initial dose). Blood samples were collected immediately before each additional dose and 0.5, 1, 2, 3, and 4 hours after each dose. After the last dose (96 hours), blood, synovial fluid, peritoneal fluid, and **bronchoalveolar lavage (BAL)** fluid were collected at various time intervals (**Table 1**). For safety reasons, foals were sedated with xylazine[c] (0.8 mg/kg, IV) prior to collection of synovial, peritoneal, and BAL fluid. Clotted blood samples as well as synovial and peritoneal fluids were centrifuged at 2,800 X g for 10 minutes. Supernatant fluid was collected and kept frozen at –70 C until assayed.

Bronchoalveolar lavage—A flexible 10-mm diameter 1.8-m bronchoscope[d] was passed via a nasal approach to the carina, and 10 ml of 2% lidocaine[e] solution was injected through the biopsy channel of the bronchoscope to minimize airway discomfort and cough. The bronchoscope was advanced until wedged in a fourth to sixth generation bronchus. The lavage solution consisted of two 100-ml aliquots of physiologic saline (0.9% NaCl) solution infused and immediately aspirated via the biopsy channel of the bronchoscope. The bronchoscope was passed into a different location (right dorsal, left dorsal, or right ventral portion of the lung) for each sample period, to minimize the effect of repeated BAL on differential cell counts. Total nucleated cell count in BAL fluid was determined by use of a hemocytometer. Slides of the BAL fluid were prepared by cytocentrifugation, and air-dried slides were stained by use of Wright-Giemsa stain. Differential count was made by examining 200 cells. Bronchoalveolar fluid was centrifuged at 200 X g for 10 minutes. Bronchoalveolar cells were washed, resuspended in 500 μl of phosphate-buffered saline solution, vortexed vigorously for 1 minute, and kept frozen at –70 C until assayed. Supernatant BAL fluid was also frozen at –70 C until assayed. Before assaying, the cell pellet samples were thawed, diluted to yield a final suspension of 2.6×10^6 cells/ml, vortexed vigorously, and sonicated for 2 minutes to ensure complete cell lysis. The resulting suspension was centrifuged at 500 X g for 10 minutes, and the supernatant fluid was used for determination of intracellular azithromycin concentrations.

Azithromycin assay—Concentrations of azithromycin were determined in serum, synovial fluid, peritoneal fluid, BAL fluid, and BAL cells, using an agar well diffusion microbiologic assay with *Micrococcus luteus*[f] as the assay organism. One milliliter of a bacterial suspension was grown overnight in trypticase soy broth and adjusted to an optical density of 0.5 at 550 nm. This suspension was added to tempered neomycin assay agar[g] and distributed evenly over the assay plates. The plates were allowed to solidify for 45 minutes, and 0.5-mm wells were punched and filled with 50 μl of samples or azithromycin standards[h] ranging in concentrations from 0.02 to 5.0 μg/ml. Known amount of purified azithromycin were added to equine serum, synovial fluid, and peritoneal fluid to produce standard curves for each type of substrate. Bronchoalveolar cells and BAL fluid were assayed with standards diluted in phosphate-buffered saline solution. The agar plates were incubated for 36 hours at 30 C. Zones of bacterial inhibition were measured to the nearest 0.1 cm. Each sample or standard was assayed in triplicate, and mean values for 3 measurements of the zone diameters were determined. The lower limit of quantitation of the assay was 0.02 μg/ml for serum, bronchoalveolar cells, and body fluid samples. Negative control samples did not cause bacterial inhibition, which indicated no antibacterial activity of equine serum, body fluids, or bronchoalveolar cell supernatants. Plots of zone diameters versus standard azithromycin concentrations were linear between 0.02 and 5 μg/ml with correlation coefficient (r) value ranging between 0.993 and 0.998. Coefficients of variation for repeatedly assayed samples at concentrations > 0.1 μg/ml and < 0.1 μg/ml were < 5% and < 10%, respectively.

Table 1—Mean ± SD azithromycin concentrations (μg/ml) after 5 intragastric doses in 6 foals given 10 mg of azithromycin/kg of body weight every 24 hours

Time (h)	Serum	Synovial fluid	Peritoneal fluid	Bronchoalveolar cells	Pulmonary epithelial lining fluid
96*	0.21 ± 0.07	—	—	—	—
96.5	0.33 ± 0.16	—	—	—	—
97	0.50 ± 0.14	—	—	—	—
98	0.58 ± 0.16	—	—	—	—
99	0.57 ± 0.09	0.34 ± 0.16	0.65 ± 0.24	89.68 ± 44.25†	11.51 ± 12.67
100	0.30 ± 0.08	—	—	—	—
104	0.33 ± 0.08	0.28 ± 0.22	0.38 ± 0.23	—	—
108	0.29 ± 0.08	—	—	—	—
120	0.23 ± 0.05	0.18 ± 0.16	0.39 ± 0.23	67.62 ± 35.70†	8.06 ± 7.71
132	0.19 ± 0.07	—	—	—	—
144	0.13 ± 0.06	—	—	89.21 ± 45.98†	6.90 ± 7.28

*Serum collected immediately before administration of azithromycin. †Drug concentrations are μg/ml of bronchoalveolar cell volume.
—= Not measured.

Estimation of PELF and BAL cell volumes and determination of azithromycin concentrations in PELF and BAL cells—Pulmonary distribution of azithromycin was determined, as reported.[13] Estimation of the volume of PELF was done by the urea dilution method.[14] Urea concentration in the BAL fluid (Urea$_{BAL}$) was determined by use of a modified enzymatic assay,[i] as described.[15,j] The time-paired plasma urea nitrogen concentrations (Urea$_{PLASMA}$) were determined by use of enzymatic methodology.[k,l] The volume of PELF (V$_{PELF}$) in BAL fluid was derived from the following equation: V$_{PELF}$ = V$_{BAL}$ × (Urea$_{BAL}$/Urea$_{PLASMA}$), where V$_{BAL}$ is the volume of recovered BAL fluid. The concentration of azithromycin in PELF (Azi$_{PELF}$) was derived from the following relationship: Azi$_{PELF}$ = Azi$_{BAL}$ × (V$_{BAL}$/V$_{PELF}$), where Azi$_{BAL}$ is the measured concentration of azithromycin in BAL fluid.

Mean volume of BAL cells (V$_{BALC}$) was determined by use of manual measurement of the diameter of 200 cells, using light microscopy of a hanging droplet. Measurement of cell volume by use of this method correlates well with velocity gradient centrifugation and morphologic studies.[3] Mean BAL cell volume obtained was 1.20 μl/10^6 cells. The concentration of azithromycin in bronchoalveolar cells (AZI$_{cells}$) was calculated, using the following relationship: Azi$_{cells}$ = (Azi$_{PELLET}$/V$_{BALC}$), where Azi$_{PELLET}$ is the concentration of antimicrobial in the BAL cell pellet supernatant.

Pharmacokinetic analysis—For each foal, 1-, 2-, and 3-component mathematical models were fit to serum concentration versus time data, using a weighted nonlinear regression analysis by a computer algorithm that minimized the sum of the squared deviations.[16] The following equations were used:

$$\text{IV: } C_t = C_1 \cdot e^{-\lambda_1 t} + C_2 \cdot e^{-\lambda_2 t} + C_3 \cdot e^{-\lambda_3 t}$$
$$\text{IG: } C_t = \Sigma_{1-5} (C_1 \cdot e^{-\lambda_1 t} + C_2 \cdot e^{-\lambda_2 t} + C_3 \cdot e^{-\lambda_3 t} - (C_1 + C_2 + C_3) \cdot e^{-\lambda_4 t})$$

where C_t is the serum drug concentration at time t for each respective dose, e is the base of the Naperian logarithm, $C_{1 \text{ to } 3}$ are preexponential and $\lambda_{1 \text{ to } 4}$ are exponential terms generated by the computer algorithm to fit the data, and λ_3 is the elimination rate constant (K_{el}). The equation for the IG data allows estimation of the area under the curve (AUC) and excretion from the data after all 5 doses. As for noncompartmental analysis, it only assumes that the drug distribution is reasonably approximated by the sum of first order processes.[17] Time (t) for each of the 5 equations is from the respective dose. The Ct values are timed from the first dose.

Elimination half-life ($t_{1/2}$) was calculated as the natural logarithm of 2 divided by K_{el}. Pharmacokinetic values were calculated on the basis of noncompartmental kinetics.[17] The AUC was calculated from the model curve, using the following equations:

$$AUC_{IV} = C_1/\lambda_1 + C_2/\lambda_2 + C_3/\lambda_3$$
$$AUC_{IG} = C_1/\lambda_1 + C_2/\lambda_2 + C_3/\lambda_3 - (C_1 + C_2 + C_3)/\lambda_4$$

The area under the first moment of the concentration-time curve (AUMC) was calculated as:

$$AUMC_{IV} = C_1/\lambda_1^2 + C_2/\lambda_2^2 + C_3/\lambda_3^2$$
$$AUMC_{IG} = C_1/\lambda_1^2 + C_2/\lambda_2^2 + C_3/\lambda_3^2 - (C_1 + C_2 + C_3)/\lambda_4^2$$

Mean residence time (MRT) was calculated from the following:

$$MRT = AUMC_{IV}/AUC_{IV}$$

Apparent volume of distribution based on the AUC (Vd$_{area}$) was calculated as follows:

$$Vd_{area} = dose_{IV}/AUC_{IV} \cdot K_{el \, IV}$$

Apparent volume of distribution at steady state (Vd$_{ss}$) was determined from:

$$Vd_{ss} = dose_{IV} \cdot AUMC_{IV}/AUC_{IV}^2$$

Clearance was calculated from dose divided by AUC and bioavailability (F) was calculated as follows:

$$F = (AUC_{IG}/AUC_{IV}) \times (dose_{IV}/dose_{IG})$$

Statistical analysis—Pharmacokinetic-derived data are presented as median and range unless otherwise specified. Azithromycin concentrations are presented as mean ± SD. The Wilcoxon sign rank test was used to compare differences in K_{el} between IV and IG administration. The paired t-test was used to compare total BAL cell counts and percentages of neutrophils, macrophages, and lymphocytes between sampling times. Differences were considered significant at $P < 0.05$.

Results

Five foals had transient adverse effects immediately after receiving the IV bolus of azithromycin, ranging from yawning (n = 5) to trembling, ataxia, and weakness (3). No adverse reactions were detected during or after repeated IG administration. After IV administration, mean ± SD serum concentration was 6.42 ± 1.72 μg/ml at 3 minutes and decreased to 0.18 ± 0.04 μg/ml by 24 hours (Fig 1). Azithromycin had $t_{1/2}$ of 20.3 hours (harmonic mean), a body clearance of 10.4 ml/min·kg, and Vd$_{ss}$ of 18.6 L/kg (Table 2).

After IG administration, detectable concentrations of azithromycin were found in 2 of 6 foals at 6 minutes and in all 6 foals at 15 minutes. The time to peak serum concentration (T_{max}) was 1.8 hours, and F was 56% (Table 2). After multiple IG administration, peak serum concentration ($C_{max \, 96 \text{ to } 144}$) was 0.63 ± 0.10 μg/ml (Fig 2). Differences between K_{el} after IV and IG administration were not significant. After multiple IG doses, peritoneal and synovial fluid concentrations were similar to serum concentrations (Table 1). The number of cells and percentage of macrophages, neutrophils, and lymphocytes in BAL fluid were not sig-
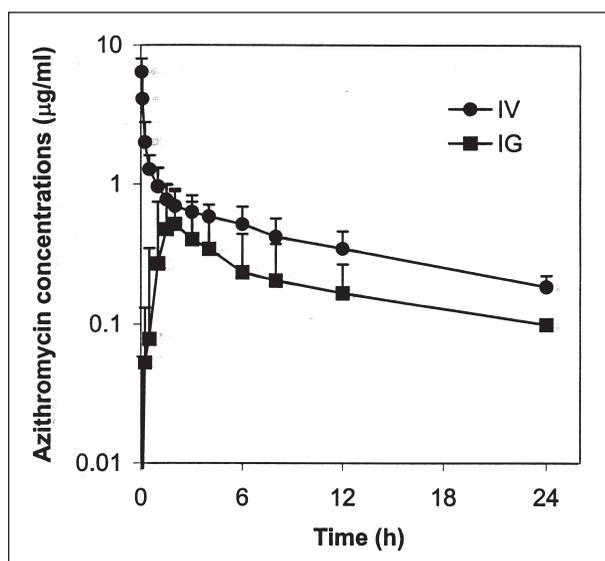

Figure 1—Mean (± SD) serum azithromycin concentrations in 6 foals administered a single IV or intragastric dose of azithromycin (10 mg/kg of body weight).

Variable	IV	Intragastric
K_{el} (h^{-1})	0.035 (0.010–0.055)	0.021 (0.013–0.042)
AUC (µg•h/ml)	16.1 (11.1–22.8)	8.1 (5.1–11.3)
AUMC (µg•h^2/ml)	361.7 (220.8–1483.8)	345.8 (147.4–675.1)
MRT (h)	23.9 (15.2–62.3)	44.0 (22.1–67.8)
$t_{1/2}$(h)	20.3 (12.6–67.4)*	NA
Vd_{area} (L/kg)	22.3 (10.6–40.8)	NA
Vd_{ss} (L/kg)	18.6 (8.8–26.2)	NA
Clearance (ml/min•kg)	10.4 (7.0–15.0)	NA
$t_{1/2abs}$ (h)	NA	0.6 (0.4–0.8)*
t_{max} (h)	NA	1.8 (1.5–3.0)
$C_{max\,0–24}$ (µg/ml)	NA	0.57 ± 0.16†
$C_{max\,96–144}$ (µg/ml)	NA	0.63 ± 0.10†
F (%)	NA	56 (28–88)

*Harmonic mean. †Mean ± SD.

K_{el} = Elimination rate constant. $t_{1/2}$ = Elimination half-life. AUC = Area under the serum concentration versus time curve. AUMC = Area under the first moment of the concentration versus time curve. MRT = Mean residence time. $t_{1/2}$ = Elimination half-life. Vd_{area} = Apparent volume of distribution (area). Vd_{ss} = Apparent volume of distribution (steady-state). $t_{1/2abs}$ = Absorption half-life. T_{max} = Time to peak serum concentration. $C_{max\,0–24}$ = Peak serum concentration after the first dose. $C_{max\,96–144}$ = Peak serum concentration after repeated doses. F = Oral bioavailability.
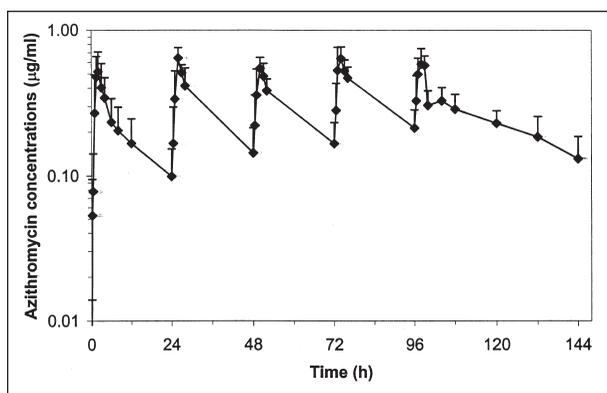

Figure 2—Mean (± SD) serum azithromycin concentrations in 6 foals administered azithromycin (10 mg/kg) intragastrically at 0, 24, 48, 72, and 96 hours.

chromatographic assays and by use of microbiologic assays.[19,20] The total antimicrobial activity measured by use of the microbiologic assay in our study was, therefore, adequate to evaluate the pharmacokinetics of azithromycin and determine a dosage regimen.

For azithromycin, mean $t_{1/2}$ in foals (20.3 hours) was shorter than that of humans (40.0 hours) but considerably longer than $t_{1/2}$ of 6.4, 7.7, and 8.7 hours reported in mice, rats, and monkeys, respectively,[2,4] and also considerably longer than values obtained for erythromycin in foals (1 hour).[8,21] In other species, the longer $t_{1/2}$ of azithromycin, compared with erythromycin, is attributed to the extensive uptake and subsequent slow release of the drug from tissues.[18] The F of azithromycin in foals (56%) was higher than values reported in humans (37%) but considerably lower than values obtained in dogs (97%).[2,4] The F of azithromycin in foals with ad libitum access to hay and water was also considerably higher than that obtained with various erythromycin formulations in foals of the same age, despite feed withholding (14.7 ± 11 to 36.4 ± 26%).[9,22] In humans, absorption of the capsular formulation and pediatric suspension of azithromycin is decreased in the presence of food, whereas the tablet formulation used in our study is equally absorbed in the fasting or fed state.[18] The effect of feeding on the F of azithromycin in foals has not been determined. There was no significant difference in K_{el} between IV or IG administration, suggesting that the route of administration does not affect distribution and elimination of the drug.

The optimal dosing of antimicrobial agents is dependant not only on the pharmacokinetics but also on the pharmacodynamics of the drug. The pharmacodynamic properties of a drug address the relationship between drug concentration and antimicrobial activity. The most important factor in determining the efficacy of some antimicrobial agents such as β-lactams is the length of time that serum concentrations exceed the MIC of the pathogen.[23] In contrast, other antimicrobial agents such as aminoglycosides and fluoroquinolones exert concentration-dependant killing characteristics.[23] Results of studies[24,25] with erythromycin indicate that the duration of time that serum concentrations exceed the MIC is the major variable that correlates with efficacy. In contrast to erythromycin, there is a poor correlation between bacterial numbers and the duration of time serum azithromycin concentrations exceed the MIC.[26] For azithromycin, the best correlation with efficacy is obtained with the AUC-to-MIC ratio, followed by the C_{max}-to-MIC ratio.[26] Therefore, the amount of drug rather than the dosing interval is the major pharmacokinetic-pharmacodynamic variable that determines the efficacy of azithromycin. In a mouse thigh model of *S pneumoniae* infection, an AUC:MIC of 200 was needed to achieve bacterial stasis, and a ratio of 800 was required to achieve bacterial killing in neutropenic animals.[26] In mice with neutrophils, the corresponding figures were 50 and 200.[26] In a *S pneumoniae* mouse peritonitis model, a C_{max}:MIC > 4 was the best predictor of outcome.[27]

Although pharmacodynamic variables based on serum concentrations may be used to estimate the opti-

nificantly different between sampling times. The majority (81.67 ± 16.21%) of the cells were macrophages. Bronchoalveolar cell and PELF azithromycin concentrations were 15- to 170-fold and 1- to 16-fold higher than concurrent serum concentrations, respectively (Table 1).

Discussion

The need for effective and potentially safer alternatives to erythromycin for the treatment of pneumonia caused by *R equi* led us to investigate the pharmacokinetics of azithromycin in foals and measure intrapulmonary steady-state concentrations of the drug, using a microbiologic assay to determine azithromycin concentrations in serum and body fluids. Microbiologic assays cannot differentiate between a drug and its active metabolites. Most of an absorbed dose of azithromycin is eliminated unchanged, principally in the feces, and no metabolite is thought to have appreciable antimicrobial activity.[5,18] As a result, there is an excellent correlation between azithromycin concentrations measured by use of high-performance liquid

mal dosage regimen of azithromycin against extracellular pathogens, they cannot be applied to the treatment of facultative intracellular pathogens such as *R equi*. Azithromycin crosses the cellular membranes primarily by use of a nonspecific diffusion mechanism.[28] It is a potent weak base that localizes in acidic intracellular compartments such as lysosomes and phagosomes. In a study[29] of the subcellular distribution of azithromycin, 50 to 70% of the drug localized in lysosomes, whereas the remaining 30 to 50% was present throughout the cell. The high and sustained intracellular concentrations achieved by azithromycin explain its in vivo efficacy against several intracellular pathogens such as *Legionella* spp, *Salmonella* spp, *Shigella* spp, and *Mycobacterium avium*, despite in vitro MIC considerably higher than achievable peak serum concentrations.[30-34] In humans, the serum protein binding of azithromycin is low and does not substantially affect the amount of drug distribution in tissues.[35] Models of localized infection with *S enteritidis*, *Escherichia coli*, *Staphylococcus aureus,* and *Bacteroides fragilis* further emphasize the importance of azithromycin concentrations at the site of infection, rather than serum concentrations, in determining the efficacy of treatment.[2,31,36]

Because serum concentrations alone could not be used to determine the likelihood of clinical efficacy in the treatment of *R equi* pneumonia of foals, azithromycin concentrations were also measured in PELF and BAL cells. Estimation of PELF volume by use of the urea dilution method may result in falsely increased BAL fluid urea concentration by diffusion of urea from the interstitium and blood if BAL fluid dwell-time is prolonged.[13] Prolonged BAL fluid dwell-time was minimized in our study by use of rapid infusion of 100 ml of saline solution followed by immediate aspiration. Overestimation of urea concentrations in BAL fluid would falsely increase the volume of PELF, which would in turn result in an underestimation of azithromycin concentrations in PELF.[13] Measurement of antimicrobial concentration in equine BAL cells had, to the authors' knowledge, never been reported. Measurement of intracellular drug concentration requires accurate measurement of cell volume. The mean BAL cell volume obtained in this study (1.2 $\mu l/10^6$ cells) was lower than values reported in humans (2.4 $\mu l/10^6$ cells) but was similar to the mean cell volume of bovine and porcine alveolar macrophages (1.28 and 1.17 $\mu l/10^6$ cells, respectively).[3,37] In the study reported here, the majority (81.67 ± 16.21%) of cells in BAL fluid were macrophages, the cell type in which *R equi* survives and replicates.[38] Concentrations of azithromycin in PELF and BAL cells considerably exceeded the MIC_{90} of 60 *R equi* isolates obtained from foals with pneumonia (1.0 $\mu g/ml$). Concentrations of azithromycin in PELF and BAL cells were still high 48 hours after administration of the last orally administered dose. In humans, sustained high concentrations and slow release from tissues allow a much shorter course of treatment, compared with other antimicrobial agents.[18,19] In contrast with PELF and bronchoalveolar cell concentrations, azithromycin concentrations in peritoneal and synovial fluids remained similar to serum concentrations in our study (Table 1).

Adverse effects in humans receiving azithromycin are rare and usually related to the gastrointestinal tract, with diarrhea, nausea, and abdominal pain being the most frequently reported.[18] In our study, a single IV bolus of azithromycin resulted in transient adverse effects characterized by yawning, trembling, ataxia, and weakness. Administration of the drug by slow IV infusion during a 1- to 3-hour period, as recommended by the manufacturer, may have attenuated or prevented these adverse effects. No adverse reactions were detected during or after repeated IG administration.

On the basis of the pharmacokinetic values, MIC of *R equi* isolates, and drug concentrations in PELF and BAL cells, a single daily oral dose of 10 mg/kg would appear appropriate for the treatment of *R equi* infections in foals. Persistence of high azithromycin concentrations in PELF and bronchoalveolar cells 48 hours after discontinuation of treatment suggests that after 5 daily doses, oral administration at 48-hour intervals may be adequate.

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