

Pharmacokinetics of tilmicosin after oral administration in swine

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Objective—To determine the pharmacokinetics of tilmicosin after oral administration of a single dose of tilmicosin base in swine.

Animals—10 healthy swine.

Procedure—Tilmicosin base was administered via stomach tube at a single dose of 20 mg/kg (n = 5) or 40 mg/kg (5). Blood samples were obtained from a jugular vein immediately before and at 10, 20, and 30 minutes and 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 hours after administration of tilmicosin. Tilmicosin concentrations in serum were quantified by use of a high-performance liquid chromatography procedure with UV light. Data for tilmicosin concentrations versus time were analyzed by use of compartmental and noncompartmental methods.

Results—Tilmicosin concentrations in serum decreased in a biexponential manner after oral administration. Mean \pm SD values for absorption half-lives were 1.49 ± 0.23 hours and 1.64 ± 0.40 hours, distribution half-lives were 2.96 ± 0.58 hours and 3.20 ± 0.76 hours, elimination half-lives were 25.26 ± 8.25 and 20.69 ± 5.07 hours, peak concentrations were 1.19 ± 0.30 $\mu\text{g/mL}$ and 2.03 ± 0.28 $\mu\text{g/mL}$, and time to peak concentrations was 3.12 ± 0.50 hours and 3.48 ± 0.77 hours after oral administration of tilmicosin base at a single dose of 20 or 40 mg/kg, respectively.

Conclusions and Clinical Relevance—In swine, tilmicosin was rapidly absorbed and slowly eliminated after oral administration of a single dose of tilmicosin base powder. (*Am J Vet Res* 2005;66:1071–1074)

Tilmicosin is a novel semisynthetic macrolide antimicrobial that has activity against *Pasteurella* spp, *Mycoplasma* spp, and various gram-positive organisms.¹ Tilmicosin has been widely used in the animal food industry in several countries for prophylactic and therapeutic purposes because of its antibacterial potency and pharmacokinetic features. Tilmicosin premix has been developed as a medicated feed additive for control of bacterial pneumonia caused by *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* in swine.²

The pharmacokinetics of tilmicosin in swine after IV administration³ and addition to the daily ration as a

medicated premix for 14 days⁴ have been investigated. The purpose of the study reported here was to investigate the pharmacokinetics of tilmicosin after oral administration of a single dose of tilmicosin base in swine.

Materials and Methods

Swine—Ten healthy 12- to 15-week-old Chester White-Yorkshire cross-bred swine weighing 23 to 31 kg were used in the study. During a 4-week acclimation period and subsequent treatment periods, swine were fed an antibacterial-free feed.^a Water was available ad libitum. Swine were housed in individual metabolism cages, which were located in a room that was isolated from all other animals. The study was approved by the Beijing Laboratory Animal Administration Committee prior to study initiation. The care and use of experimental animals complied with local animal welfare laws, guidelines, and policies.

Tilmicosin administration—Food was withheld from swine for 10 to 12 hours prior to administration of tilmicosin base powder.^b Tilmicosin was administered to 5 swine at a dose of 20 mg/kg. This dose was chosen on the basis of the United States approved daily dose^c of 200 to 400 mg/kg (which equals 181.8 to 363.6 g of tilmicosin phosphate/ton of feed and results in a tilmicosin consumption of approx 10.97 to 21.34 mg/kg per pig per day). To confirm linear pharmacokinetics, an additional dose of 40 mg/kg was also examined in a separate group of 5 swine. To facilitate dosing, the mouth was held open with a speculum and a stomach tube was passed. The tilmicosin base powder was mixed with tap water into a gruel-like consistency. After the dose was administered, the stomach tube was flushed with approximately 50 mL of clean tap water.

Sample collection—Blood samples were obtained from the jugular vein immediately before and at 10, 20, and 30 minutes and 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 hours after dosing. Blood samples were stored at 4°C and permitted to clot. The serum was obtained after centrifugation for 15 minutes at 756 \times g and stored at -20°C. Blood and serum samples were protected from direct light because of the known photosensitivity of tilmicosin.

Determination of tilmicosin concentrations—Tilmicosin concentrations in serum were quantified by use of high-performance liquid chromatography^d (HPLC) with UV detector. The analytical method for the determination of tilmicosin in serum was modified from the method developed by John et al.⁵

Serum samples were prepared for HPLC analysis with solid-phase extraction cartridges.^d The cartridge was activated with 10 mL of methanol and 10 mL of deionized water prior to addition of 2 mL of serum. After the serum was drained through the cartridge, the cartridge was washed with 10 mL of deionized water, then with 10 mL of 35% acetonitrile in deionized water. After the washing solution had been drained, the cartridge was dried at high vacuum for at least 5 minutes.

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Tilmicosin was eluted from the cartridge with 2.5 mL of 0.1M ammonium acetate in a solution of methanol and acetonitrile (vol/vol, 20:80). The collected eluent was evaporated to dryness under a nitrogen stream and reconstituted in 1 mL of the mobile-phase solution prepared as below. After filtering through a 0.2 µm polytetrafluoroethylene filter,^c the reconstituted samples were injected into the HPLC system.

The mobile-phase solution was prepared by adding 135 mL of acetonitrile, 55 mL of tetrahydrofuran, and 25 mL of 1M dibutylammonium phosphate buffer to 700 mL of water and diluting with additional water to obtain a final volume of 1,000 mL. The 1M dibutylammonium phosphate buffer was prepared by adding 70 mL of 10% phosphoric acid in water (vol/vol) to 16.8 mL of dibutylamine, allowing it to cool, adjusting with phosphoric acid to a pH of 2.5 ± 0.1, and diluting with water to 100 mL. The flow rate was 1.0 mL/min. The run time was 20 minutes. The detector wavelength was set at 290 nm.

Tilmicosin base reference standard^d stock solution (2,000 µg/mL) was prepared by dissolving dried tilmicosin compound in acetonitrile. The stock solution was stable for 3 months when refrigerated in a light-protected environment.

A calibration curve of peak area (both cis- and trans-isomers of tilmicosin) versus concentration of the tilmicosin reference standard was constructed with at least 4 and as many as 6 concentrations. The calibration curve was found to be linear in the range of 0.025 to 25 µg/mL, and the correlation coefficient (*R*) was ± 0.9997. The calculated limit of detection for the method was 0.0125 µg/mL on the basis of a signal-to-noise ratio of 3:1. The **limit of quantitation (LOQ)** in swine serum was 0.025 µg/mL on the basis of a signal-to-noise ratio of 6:1.

Fortified at concentrations of 0.025, 0.5, and 5 µg/mL, recoveries of tilmicosin in serum were 104.5%, 91.4%, and 94.6%, respectively, as determined by comparing the peak areas of tilmicosin (cis and trans) in serum samples with the peak areas of tilmicosin in the working standard solution. The coefficients of variation ranged from 4.3% to 5.5%.

Statistical analyses—A nonlinear least squares regression analysis program⁸ was used to fit the serum concentration-time data to a series of pharmacokinetic models with serum tilmicosin concentration versus time data weighted by 1, 1/*c*, and 1/*c*², where *c* is the serum tilmicosin concentration. The concentration versus time data for tilmicosin were obtained from each animal and used to calculate kinetic disposition. On the basis of comparisons of **Akaike information criterion (AIC)** values and the range of the coefficient of determination (*R*²) values in 1-, 2-, and 3-compartment open models, the number of exponential terms was determined.^{6,7} Further pharmacokinetic analysis was performed by use of a noncompartmental method of statistical moment theory⁸ to calculate the **area under the curve from zero to infinity (AUC)**, **area under the first-moment curve from zero to infinity (AUMC)**, and **mean residence time (MRT)**. Computer software⁹ was used for the statistical analysis of pharmacokinetic values. A paired 2-tailed Student *t* test was used to determine significant differences in pharmacokinetic parameters between the 2 dose groups after oral administration of tilmicosin. A value of *P* < 0.05 was considered significant.

Results

No adverse effects were observed in any swine after tilmicosin administration. The concentrations of tilmicosin in swine serum were determined for 120 hours after oral administration. When the serum concentrations of tilmicosin were less than the LOQ

(0.025 µg/mL), the pharmacokinetic analysis was based on the time associated with the last quantifiable tilmicosin concentration (which generally was 72 hours after administration).

Mean ± SD tilmicosin concentrations in serum at each collection time after oral administration of tilmicosin base and corresponding plots of the predicted mean concentration versus time profiles were determined (Table 1; Figure 1). Weighted by 1/*c*, the pharmacokinetic analysis was performed on the basis of the serum tilmicosin concentrations for each individual animal at each time point. Comparison of AIC and *R*² values in 1-, 2-, and 3-compartment models suggested that the data best fit a 2-compartment open model, which had the smallest AIC and the largest *R*² (0.905 to 0.987) values. Therefore, the compartmental analysis was based on the use of the following biexponential equation:

$$C_{\text{serum}} = A e^{-\alpha t} + B e^{-\beta t} - (A + B) e^{-K_{at}}$$

where *C*_{serum} is the concentration of tilmicosin in serum at time *t*; *A* and *B* are the intercepts for the distribution and elimination phases (determined by extrapolation to the *y*-intercepts); *e* is the exponent; and *K*_a, *α*, and *β* are the absorption, distribution, and elimination rate constants, respectively.

Absorption and distribution phases occurred simultaneously. Therefore, the distribution phase described in the analysis represents tilmicosin that is being distributed out of a deep compartment into the blood. Estimates of *K*_a are tenuous at best because of the absence of data collected after IV administration of tilmicosin, which could not be obtained because of the known cardiotoxicity associated with IV administration of tilmicosin to swine.

Mean ± SD values for AUC (14.01 ± 2.25 µg·h/mL and 29.41 ± 3.37 µg·h/mL) were proportional to the doses of tilmicosin (20 and 40 mg/kg) administered (Table 2). The dose of tilmicosin administered also had a minimal effect on the values of *K*_a, *α*, *β*, and **time to peak concentration (t_{max})**, as was confirmed by the ratio of parameter values associated with the 20 and 40 mg/kg doses; ratios for *K*_a, *α*, *β*, and *t*_{max} were 1.07, 1.04, 0.75, and 0.89, respectively. Accordingly, the effect of dose on

Table 1—Mean ± SD concentrations (µg/mL) of tilmicosin in serum at various time points after administration of a single dose of tilmicosin (20 and 40 mg/kg, PO) in swine (*n* = 5 for each dose group).

Time (h)	20 mg/kg	40 mg/kg
0.167	0.02 ± 0.01	0.05 ± 0.01
0.333	0.13 ± 0.06	0.25 ± 0.07
0.5	0.25 ± 0.10	0.46 ± 0.15
1	0.58 ± 0.15	1.24 ± 0.21
2	1.34 ± 0.48	2.20 ± 0.22
3	1.55 ± 0.30	2.99 ± 0.28
4	1.33 ± 0.28	2.12 ± 0.18
6	0.89 ± 0.20	1.61 ± 0.21
8	0.61 ± 0.07	1.23 ± 0.21
12	0.38 ± 0.11	0.85 ± 0.15
24	0.13 ± 0.05	0.32 ± 0.06
36	0.07 ± 0.03	0.17 ± 0.03
48	0.05 ± 0.01	0.10 ± 0.02
72	0.02 ± 0.00	0.04 ± 0.01

these parameter values was not significant. Tilmicosin administered at doses of 20 and 40 mg/kg, PO, was rapidly absorbed with mean \pm SD absorption half-lives of 1.49 ± 0.23 hours and 1.64 ± 0.40 hours, respectively, and it distributed with mean \pm SD half-lives of 2.96 ± 0.58 hours and 3.20 ± 0.76 hours, respectively, for the distribution phase. Tilmicosin was slowly eliminated from the central compartment, with mean \pm SD half-lives of 25.26 ± 8.25 hours and 20.69 ± 5.07 hours for the elimination phase. The mean \pm SD peak concentra-

tions of tilmicosin in serum (1.19 ± 0.30 and 2.03 ± 0.28 $\mu\text{g/mL}$) were reached 3.12 ± 0.50 hours and 3.48 ± 0.77 hours, respectively, after administration of tilmicosin at doses of 20 and 40 mg/kg, PO.

The AUC, AUMC, and MRT values were also obtained via noncompartmental methods to assess the magnitude of bias in the pharmacokinetic exposure estimates that was attributable to model misspecification (Table 3). On average, AUC values obtained via noncompartmental methods were 30% larger than those estimated on the basis of fitted pharmacokinetic compartmental parameter values.

Discussion

Tilmicosin is approved as a feed medication for control of respiratory disease associated with *A pleuropneumoniae* and *P multocida* in swine. Classical pharmacokinetic parameters have not been generated for tilmicosin in swine because of the toxicity associated with IV administration. For the same reason, the bioavailability of tilmicosin after oral administration to swine has not been determined. Tilmicosin administered to swine as an IV bolus at a dose of 4.5 mg/kg caused considerable adverse cardiovascular effects and death.³

Results of our study indicated that data for serum concentration versus time generated after oral administration of tilmicosin were best fitted to a 2-compartment open model. When administering tilmicosin orally, the absorption and distribution phases occur simultaneously. The distribution phase represents tilmicosin that is distributing out of a deep compartment into the blood, where it is subsequently eliminated. The absorption half-lives of tilmicosin after administration at doses of 20 and 40 mg/kg, PO, were 1.49 ± 0.23 hours and 1.64 ± 0.40 hours, respectively. The elimination half-lives for each dose were > 20 hours, and the AUC was > 1.0 $\mu\text{g}\cdot\text{h/mL}$. Results indicated that tilmicosin is quickly absorbed and slowly eliminated after oral administration in swine.

At present, there are few reports on the pharmacokinetics of tilmicosin after oral administration to animals. Thomson et al⁴ previously determined tilmicosin concentrations in the serum of swine after administration of 200 and 400 mg of tilmicosin/kg in feed. In that study, postmortem serum samples were collected from groups of 4 swine (2 male and 2 female) that were serially slaughtered at 2, 4, 7, 10, and 14 days after the initiation of treatment. The investigators observed that the tilmicosin concentrations in serum were less than the LOQ (0.1 mg/L) in 17 of 20 swine administered the 200 mg/kg dose. At the 400 mg/kg dose, tilmicosin concentrations in serum ranged from < 0.1 to 0.23 mg/L, with detectable concentrations in 17 of 20 swine. Both dose groups had serum tilmicosin concentrations that were lower than those seen in the study reported here after administration of tilmicosin via oral gavage. Keles et al⁸ investigated the pharmacokinetics and tissue concentrations of tilmicosin in fowl. After oral administration of a single dose of tilmicosin (50 mg/kg), the mean maximum concentration of tilmicosin in the lungs was 6.2 times greater than that observed in serum. The total systemic clearance was

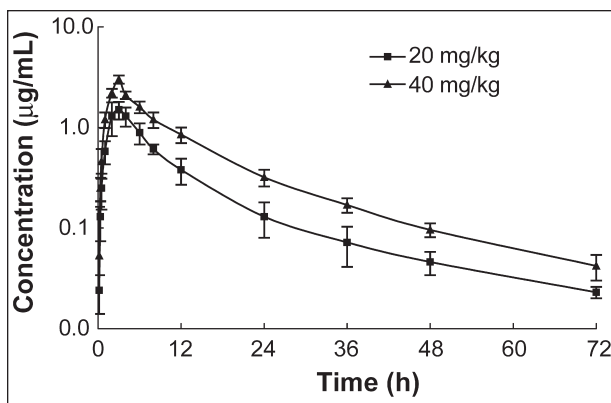


Figure 1—Mean \pm SD concentration versus time curves for tilmicosin in serum after administration of a single dose of tilmicosin base (20 or 40 mg/kg, PO) in swine ($n = 5$ in each dose group).

Table 2—Mean \pm SD values for pharmacokinetic parameters after administration of a single dose of tilmicosin (20 and 40 mg/kg, PO) in swine ($n = 5$ for each dose group).

Parameters	20 mg/kg	40 mg/kg
A ($\mu\text{g/mL}$)	4.69 ± 1.94	7.16 ± 1.27
B ($\mu\text{g/mL}$)	0.17 ± 0.10	0.55 ± 0.17
Ka (L/h)	0.47 ± 0.07	0.44 ± 0.10
α (L/h)	0.24 ± 0.04	0.23 ± 0.05
β (L/h)	0.03 ± 0.01	0.04 ± 0.01
$t_{1/2Ka}$ (h)	1.49 ± 0.23	1.64 ± 0.40
$t_{1/2\alpha}$ (h)	2.96 ± 0.58	3.20 ± 0.76
$t_{1/2\beta}$ (h)	25.26 ± 8.25	20.69 ± 5.07
AUC ($\mu\text{g}\cdot\text{h/mL}$)	14.01 ± 2.25	29.41 ± 3.37
t_{max} (h)	3.12 ± 0.50	3.48 ± 0.77
C_{max} ($\mu\text{g/mL}$)	1.19 ± 0.30	2.03 ± 0.28

A = Intercept for the distribution phase. B = Intercept for the elimination phase. Ka = Absorption rate constant. α = Distribution rate constant (the distribution phase represents drug that is being distributed out of a deep compartment into the blood). β = Elimination rate constant. $t_{1/2Ka}$ = Half-life of the absorption phase. $t_{1/2\alpha}$ = Half-life of the distribution phase. $t_{1/2\beta}$ = Half-life of the elimination phase. AUC = Area under the curve from zero to infinity. t_{max} = Time to peak concentration. C_{max} = Peak concentration.

Table 3—Mean \pm SD values for pharmacokinetic parameters obtained by use of a noncompartmental method after administration of a single dose of tilmicosin (20 and 40 mg/kg, PO) in swine ($n = 5$ for each dose group).

Parameters	20 mg/kg	40 mg/kg
AUC ($\mu\text{g}\cdot\text{h/mL}$)	18.27 ± 3.01	32.36 ± 0.07
AUMC ($\mu\text{g}\cdot\text{h}^2/\text{mL}$)	330.73 ± 72.77	545.53 ± 61.39
MRT (h)	17.81 ± 1.16	16.79 ± 1.18

AUC = Area under the curve from zero to infinity. AUMC = Area under the first-moment curve from zero to infinity. MRT = Mean residence time. See Table 2 for remainder of key.

1.33 L/h. Tilmicosin was eliminated more slowly from the lungs (mean half-life, 75.74 hours) than from serum (30.18 hours) in fowl.

In the study reported here, the mean peak concentrations detected in serum after oral administration of tilmicosin at single doses of 20 and 40 mg/kg were $1.19 \pm 0.30 \mu\text{g/mL}$ and $2.03 \pm 0.28 \mu\text{g/mL}$, which were lower than the minimum inhibitory concentrations (MICs) for *Actinobacillus suis* (8 $\mu\text{g/mL}$) and *P. multocida* type A (8 $\mu\text{g/mL}$) isolated clinically from swine.⁹ Similar MICs were reported by Blackall et al.¹⁰ and Salmon et al.¹¹ Thomson et al.⁴ determined the tilmicosin concentration in lung tissues of swine. When tilmicosin was fed to swine at doses of 200 and 400 mg/kg for 2, 4, 7, 10, and 14 days, tilmicosin concentrations in lung tissues ranged from 0.73 to 1.43 $\mu\text{g/mL}$ and 1.11 to 2.59 $\mu\text{g/mL}$, respectively, both of which are also lower than the MICs for *A. suis* and *P. multocida*. Nevertheless, results of clinical field trials^{12,13} confirm that tilmicosin administered in feed at a dose of 200 mg/kg is effective for control of pneumonia in swine attributable to *A. pleuropneumoniae* or *P. multocida*.

Reasons for efficacy against *A. pleuropneumoniae* and *P. multocida*, despite low to nondetectable concentrations of tilmicosin in serum and lung tissues, were explored via a review of the literature. Modric et al.¹⁴ reported that concentrations of tilmicosin in the lungs were significantly higher than concentrations of tilmicosin in serum at all times tested and that mycoplasma-infected rats had significantly higher concentrations of tilmicosin in lung tissues than noninfected rats. Shryock and Scorneaux et al.¹⁵ determined the in vitro uptake of tilmicosin by alveolar macrophages by incubating these cells with 10 and 20 $\mu\text{g/mL}$ of tilmicosin at 37°C for 4 hours. Results of that study indicated that tilmicosin readily accumulates in swine alveolar macrophages, with a high ratio of intracellular or extracellular tilmicosin concentrations. Scorneaux and Shryock et al.¹⁶ reported that tilmicosin accumulates in the bovine alveolar macrophage. The fact that tilmicosin accumulates substantially in various swine phagocytes, even in the presence of low extracellular concentrations, provides insight as to why tilmicosin is effective for the control of bacterial pneumonia in swine.^{4,15} On the basis of information contained in those studies, the concentration of tilmicosin in lung tissues would be expected to be higher than that in serum, with possibly even higher concentrations in the alveolar macrophages located at the site of infection. This hypothesis warrants further investigation.

- a. Complete Feed, Feed Institute, China Agriculture Academy, Beijing, PR China.
- b. Tilmicosin powder, Jining Veterinary Medicine Corp, Shandong Province, PR China.
- c. Waters HPLC system, Waters Corp, Milford, Mass.

- d. Bon Elut cartridge, Varian China Ltd, Beijing, PR China.
- e. PTFE filter, Varian China Ltd, Beijing, PR China.
- f. Tilmicosin standard, Lilly Research Laboratories, Indianapolis, Ind.
- g. 3P97 program, Chinese Pharmacological Society, Beijing, PR China.
- h. SPSS software, version 10.0, SPSS Inc, Chicago, Ill.

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