Pharmacokinetics of Tilmicosin® in Crossbred Pigs Following Single Oral Administration

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Introduction
Tilmicosin is a semi-synthetic macrolide antibiotic used abundantly in veterinary medicine. It possesses excellent in vitro activity against gram-positive bacteria, Mycoplasmaspp., as well as certain gram-negative bacteria (2). In addition, a recent study reported that tilmicosin also exhibits strong antiviral effects against PRRS virus by altering endosomal pH after administration (4). Tilmicosin rapidly distributes to lung tissue and can subsequently be accumulated in alveolar macrophages (7). Tilmicosin used in pigs can effectively control and treat respiratory diseases caused by Actinobacillus pleuropneumoniae, Pasteurellamultocida, Mycoplasma hyopneumoniae and other microbes (5). In field practice, uses of tilmicosin to control bacterial infection in pigs has been administered via medicated feed. Its recommended dosages for controlling and preventing Actinobacillus pleuropneumoniae infection were 200 or 400 mg/kg feed, respectively, for 21 consecutive days. Several pharmaceutical techniques have currently been developed to improve tilmicosin oral absorption including granulation technique. The purpose of this study was to determine pharmacokinetics of Resmitil® (Granulated form of 20% tilmicosin phosphate premix) after single oral administration in crossbred pigs.

Materials & Methods
Animals: Six healthy castrated male crossbred pigs at 8-9 weeks old (~25 kg bw) were used. Antibiotic-free pelleted feed and water was provided ad libitum. They were acclimatized for 14 days at the laboratory animal facility, the Department of Animal Science, Faculty of Agriculture, Kasetsart University, Kamphangsaen Campus.

Tilmicosin Administration: Resmitil® (20% premix) containing 200 mg tilmicosin per 1 g premix was administered at the dosage of 16 mg/kg bw to all 6 pigs.

Plasma Collection: Blood samples were obtained via jugular vein and placed in heparinized tubes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36 and 48 hours after administration. Each blood sample was centrifuged at 3500 rpm for 15 minutes, plasma was collected, and then kept at -80°C until analysis.

Sample Extraction: Plasma samples were added with 200 µL of 25 µg/mL of tyllosinan internal standard followed by 2 mL of acetonitrile (ACN), then vortex mixed for 1 minute and centrifuged at 4000 rpm for 20 min (~4°C). The supernatant was collected and applied onto a C18 SPE cartridge (Sep-Pak C18, Waters). The SPE cartridge was eluted 2.5 ml of 0.1 M ammonium acetate in MeOH:ACN (20:80). The collected eluent was evaporated under nitrogen stream. It was reconstituted with 250 µL of 0.1 M ammonium acetate in MeOH:ACN (20:80) and filtered with 0.2 µm PTFE filter before injecting into a UPLC system.

Determination of Plasma Concentration by UPLC: The LC instrument used in this experiment was ACQUITY UPLC H-Class System (Waters). The chromatographic column was a reversed phase column (Acquity C18, 1.7µM, 2.1 mm x 100). The analysis was performed using gradient mode with 0.05% Trifluoroacetic acid/TFA and acetonitrile (HPLC grade) as mobile phases. The flow rate was 0.4 mL/min with total run time of 10 minutes. The analytes were detected at 290
nm wavelength. The analysis was validated for the determination of tilmicosin in plasma by using tylosin as an internal standard. The chromatogram was shown in Figure 1.

![Chromatogram](image)

**Figure 1** Chromatogram of the analyte (Tilmicosin) and an internal standard (Tylosin) in plasma

**Data Analysis** The pharmacokinetic analysis of the data was performed using PK Solution 2.0™ Program (Summit Research Services, Montrose, CO, U.S.A.). The relevant pharmacokinetic parameters were recalculated. All data are expressed as mean ± standard error of the mean (SEM).

**Results and Discussion**

No adverse effects were observed in pigs after tilmicosin (Resmitil®) administration in this experiment. Following single oral administration of granulated tilmicosin formulation at the dosage of 16 mg/kg bw, pig plasma concentrations were individually reported (Figure 2). The pharmacokinetic parameters were summarized in Table 2.

A previous tilmicosin study following single oral administration at 20 and 40 mg/kg bw showed mean peak plasma concentrations of 1.19 ± 0.30 and 2.03 ± 0.28 µg/mL with the time to peak concentration at 3.12 ± 0.50 hr and 3.48 ± 0.77 hr, respectively (7). In our study, the

![Individual tilmicosin plasma concentrations](image)

**Figure 2** Individual tilmicosin plasma concentrations after single oral administration in pigs with tilmicosin (Resmitil®) at a dosage of 16 mg/kg bw

**Table 2** Pharmacokinetic parameters of tilmicosin (Resmitil®) after single oral administration in pigs at a dosage of 16 mg/kg bw. Higher Cmax and the lower Tmax than in Shen's study were apparent. Differences in dosages actually play an important role. Another study at the same dosage (16 mg/kg) showed similar Tmax (2.1 ± 0.1 hr) to our study (3). However, our peak concentration level was relatively higher (2.54 ± 0.15 compared to 1.03 ± 0.03 µg/mL). In addition, the mean elimination half-life (t1/2) in this study was 23.5 ± 4.7 hr whereas pigs received 20 mg/kg bw of tilmicosin was 25.26 ± 8.25 hr (7).

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Units</th>
<th>Mean ± SEM</th>
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<tbody>
<tr>
<td>Cmax</td>
<td>µg/mL</td>
<td>2.54 ± 0.15</td>
</tr>
<tr>
<td>Tmax</td>
<td>hr</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>t1/2</td>
<td>hr</td>
<td>23.5 ± 4.7</td>
</tr>
<tr>
<td>AUC(0-t)</td>
<td>µg-hr/mL</td>
<td>16.3 ± 0.80</td>
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<tr>
<td>AUC∞</td>
<td>µg-hr/mL</td>
<td>20.4 ± 1.36</td>
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<tr>
<td>AUMC∞</td>
<td>µg-hr*hr/mL</td>
<td>703.6 ± 156.9</td>
</tr>
<tr>
<td>MRT</td>
<td>hr</td>
<td>27.9 ± 4.4</td>
</tr>
<tr>
<td>CL</td>
<td>mL/hr/kg</td>
<td>896.3 ± 59.2</td>
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**Conclusion**

The granulated form of tilmicosin (Resmitil®) after a single oral administration in pigs was rapidly absorbed and slowly eliminated resulting in longer period of detectable plasma concentration from 30 minutes to at least 48 hr. Maximal concentration (2.54 ± 0.15 µg/mL) in plasma was observed at 21 ± 0.1 hr after administration.
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References