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Pharmacokinetics of gamithromycin after intravenous and subcutaneous administration in pigs

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ABSTRACT

The aim of this study was to investigate the pharmacokinetic properties of gamithromycin in pigs after an intravenous (i.v.) or subcutaneous (s.c.) bolus injection of 6 mg/kg body weight. The plasma concentrations of gamithromycin were determined using a validated high-performance liquid chromatography–tandem mass spectrometry method, and the pharmacokinetics were noncompartmentally analysed.

Following i.v. administration, the mean area under the plasma concentration–time curve extrapolated to infinity (AUC_{inf}) and the mean elimination half-life ($t_{1/2\lambda z}$) were 3.67 ± 0.75 μg.h/mL and 16.03 h, respectively. The volume of distribution at steady state (V_{ss}) and the plasma clearance were 31.03 ± 6.68 L/kg and 1.69 ± 0.33 L/h.kg, respectively. The mean residence time (MRT_{inf}) was 18.84 ± 4.94 h.

Gamithromycin administered subcutaneously to pigs demonstrated a rapid and complete absorption, with a mean maximal plasma concentration (C_{max}) of 0.41 ± 0.090 μg/ml at 0.63 ± 0.21 h and a high absolute bioavailability of 118%.

None of the reported pharmacokinetic variables significantly differed between both administration routes.

Macrolide antibiotics are classified as macrocyclic lactone rings containing 12–20 carbon atoms with diverse combinations of deoxy sugars attached to this ring by glycosidic linkages. The antibacterial action of macrolides comprises the inhibition of protein synthesis by binding to the 50S ribosomal subunit of prokaryotic organisms and consequently inhibiting the translocation (Papich and Riviere, 2009).

In addition to the anti-infectious properties, these drugs have been reported to influence a variety of inflammatory processes, such as the release of cytokines and mediators, the migration of neutrophils and the oxidative burst in phagocytes. A significant decrease of the production of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 was reported after *in vitro* lipopolysaccharide (LPS) stimulation and treatment with tilmicosin and tylosin. Moreover, macrolides are also characterised by an extensive accumulation into leukocytes and lung tissues, achieving much higher tissue concentrations compared to those observed in plasma (Nightingale, 1997; Ianaro et al., 2000; Cao et al., 2006; Tauber and Nau, 2008; EMA, 2008a; Buret, 2010).

Via introduction of a nitrogen atom into the macrolactone ring, a novel class of macrolide antibiotics, the so-called azalides, was generated. Azythromycin was the first azalide which has been associated with remarkable pharmacokinetics, such as a high tissue distribution, metabolic stability and a high tolerability compared to other macrolide antibiotics (Mutak, 2007).

In veterinary medicine, gamithromycin is a 15-membered semisynthetic macrolide antibiotic of the azalide subclass which has been recently developed for the treatment and prevention of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* (Huang et al., 2010).

Bacteria, such as *Actinobacillus pleuropneumoniae*, *P. multocida*, *Haemophilus parasuis* and *Mycoplasma hyopneumoniae* are major pathogens involved in swine respiratory disease (SRD). In growing pigs, respiratory infections are responsible for severe economical losses and reduced animal welfare. Other second generation macrolide antibiotics, such as tulathromycin (Draxxin®) and tildipirosin (Zuprevo®), have been approved for treatment of SRD in pigs (EMA, 2008a, 2011; Rose et al., 2013).

The aim of this study was to determine the PK properties of gamithromycin in pigs, whereafter the characteristics of this antibiotic can be used in future research to investigate the immunomodulatory properties in an *in vitro* and *in vivo* porcine LPS inflammation model (Wyns et al., 2013).

Twelve clinically healthy male pigs (Landrace) with a mean body weight (BW) of 24.81 ± 1.65 kg were randomly divided in two groups. The study was conducted according to a single dose

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parallel design. The pigs received a bolus injection of 6 mg/kg BW gamithromycin (Zactran®, Merial), either intravenously (i.v.) in the ear vein ($n = 6$) or subcutaneously (s.c.) in the flank region ($n = 6$). Blood was collected by venepuncture of the jugular vein into EDTA-coated tubes (Vacutest Kima) before administration (time 0 h); at 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 10, 12 h post administration (p.a.) and once daily from day 2 to day 14 p.a. Blood samples were centrifuged and plasma was stored at $\leqslant -15$ °C until analysis.

The animal experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University (EC2011/159).

After a solid phase extraction, using HybridSPE®-Phospholipid cartridges, the quantification of gamithromycin in porcine plasma was performed using a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) method, which was previously described in detail by Watteyn et al. (2013).

The PK properties were noncompartmentally determined by means of WinNonlin®, version 6.2.0 software program (Pharsight Corporation). Values below the limit of quantification of 5 ng/mL were not included in the analysis. The mean area under the plasma concentration–time curve was calculated using the linear trapezoidal method from time 0 to the last time point with a quantifiable concentration (AUC_{last}) and the AUC extrapolated to infinity (AUC_{inf}). The absolute bioavailability (F) was calculated from the following equation:

$$F(\%) = \frac{AUC_{0\rightarrow\infty s.c.}}{AUC_{0\rightarrow\infty i.v.}} \times 100$$

The data are presented as mean ± standard deviation (SD), except for F, and were statistically analysed by means of single-factor analysis of variance (ANOVA), using SPSS Version 20.0 software for Windows. The level of significance was $\alpha = 0.05$.

No adverse effects following i.v. or s.c. administration were observed during the course of the study. Semi-logaritmic plots of the mean plasma concentration–time curves following i.v. and s.c. administration are presented in Fig. 1. Quantifiable concentrations of gamithromycin in plasma were present for 48 and 72 h after i.v. and s.c. administration, respectively. The PK properties of gamithromycin (mean ± SD) are summarized in Table 1.

Following an i.v. bolus injection of 6 mg/kg BW gamithromycin, the AUC_{inf} was 3.67 ± 0.75 μg.h/mL. The elimination half-life ($t_{1/2\lambda z}$) was 16.03 h. The volume of distribution at steady state (V_{ss}) and clearance (Cl) were 31.03 L/kg and 1.69 ± 0.33 L/h.kg, respectively. The mean residence time extrapolated to infinity (MRT_{inf}) was 18.84 ± 4.94 h. The volume of distribution at steady state (V_{ss}) was 31.03 ± 6.68 L/kg.

Following a s.c. bolus injection of 6 mg/kg BW gamithromycin, the AUC_{inf} was 4.31 ± 1.14 μg.h/mL. A maximal plasma concentration (C_{max}) of 0.41 ± 0.090 μg/mL was reached at 0.63 ± 0.21 h (t_{max}). The $t_{1/2\lambda z}$ and MRT_{inf} were 18.76 and 24.41 ± 9.17 h, respectively. The absolute bioavailability of gamithromycin was 117.6%.

None of the reported pharmacokinetic properties significantly differed between both administration routes.

In the current study in pigs, a higher clearance of gamithromycin was observed compared to cattle (1.69 vs 0.71 L/h.kg), resulting in a shorter $t_{1/2\lambda z}$ (16.03 vs 44.9 h in pigs and cattle, respectively) after i.v. administration of gamithromycin. The V_{ss}, on the other hand, was quite comparable between both species (31.03 and 24.90 L/kg, respectively) (Huang et al., 2010). Notwithstanding a generally more pronounced metabolic rate in birds, similar values for clearance and $t_{1/2\lambda z}$ (1.61 L/h.kg and 14.12 h, respectively) were recently observed in broiler chickens (Watteyn et al., 2013). In addition, the V_{ss} was high in both species (31.03 and 29.16 L/kg in pigs and broiler chickens, respectively). Macrolide antibiotics are lipophilic molecules and are subsequently well absorbed and extensively distributed in body fluids and tissues (Zuckerman et al., 2011). Conversely, binding to plasma proteins can considerably restrict this extravascular distribution (Huang et al., 2010). In pigs, the mean plasma protein binding of gamithromycin was only 23% (EMA, 2008b).

In comparison with other macrolide antibiotics, gamithromycin showed a relatively short $t_{1/2\lambda z}$. The clearance of gamithromycin was considerably higher than that of tulathromycin (0.58 L/h.kg, Benchaoui et al., 2004 and 0.18 L/h.kg, Wang et al., 2011). In this respect, tulathromycin, approved for treatment of bacterial SRD, showed a $t_{1/2\lambda z}$ of 67.5–76.5, 75.6 and 78.7 h after an i.v., i.m. and oral bolus of 2.5 mg/kg BW, respectively (Benchaoui et al., 2004; Wang et al., 2011). Likewise, tildipirosin, a semi-synthetic tylosin analogue also approved for SRD, revealed a very slow elimination with a $t_{1/2\lambda z}$ of 97 h following a single i.m. injection of 6 mg/kg BW (Rose et al., 2013). On the other hand, tylosin, a first generation macrolide antibiotic mainly used to treat pneumonia and dysentery in pigs, has a $t_{1/2\lambda z}$ of 4.52 and 24.5 h after i.v. and i.m. administration of 10 mg/kg BW, respectively. The total body clearance of tylosin is comparable to that of gamithromycin (1.88 L/h.kg, Prats et al., 2002). While the short $t_{1/2\lambda z}$ of tylosin after i.v. administration

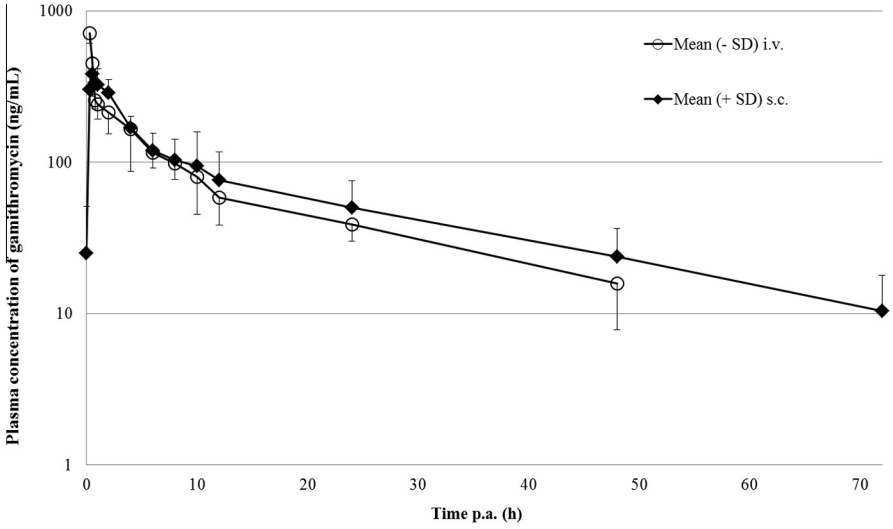

Fig. 1. Mean (± SD) plasma concentration–time profiles of gamithromycin after i.v. ($n = 6$) and s.c. ($n = 6$) bolus administration of 6 mg/kg BW in pigs.