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# Pharmacokinetics of tildipirosin in pig tonsils

F. TORRES\*

R. SANTAMARIA†

M. JIMENEZ‡

R. MENJÓN†

A. IBANEZ‡

M. COLLELL§

AZLOR§ &

FRAILE\*¶

\*Departament de Producció Animal, ETSEA, University de Lleida, Lleida, Spain; †Technical Service MSD Animal Health, Madrid, Spain; ‡Marketing Manager MSD Animal Health, Madrid, Spain; §MSD Global Swine, Summit, NJ, USA; ¶Agrotecnio Center, Lleida, Spain

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The penetration of antimicrobials in pig tonsils is hardly known. The objective of the study was to quantify the tildipirosin (TD) penetration in tonsils. Animals were randomly divided into six groups (control, T1, T2 (1), T2(5), T2(10), and T2(15)) of eight animals. T1 and T2 groups received a dose of 2 and 4 mg of TD/kg bw in one shot (Zuprevo® MSD Animal Health), respectively, and the control group received 2 mL of saline solution. The animals were sacrificed by intravenous administration of pentobarbital sodium 24 h after finishing the treatment for the control, T1, and T2(1) groups, whereas animals of T2(5), T2(10), and T2(15) groups were sacrificed at 5, 10, and 15 days, post-treatment, respectively. Tonsils and blood samples were taken at necropsy to obtain plasma, and the tildipirosin concentration was determined by high-performance liquid chromatography with tandem mass spectrometry detection. The concentration in plasma was always significantly lower than in tonsil. Average TD tonsil concentrations increased significantly in a dose-dependent manner, and the tonsil TD vs. plasma TD concentration ratio was approximately 75 for the doses of 2 and 4 mg of TD/kg bw at 24 h post-treatment. Moreover, the maximum concentration of tildipirosin in tonsil was observed at 1 day postadministration, and this concentration decreased gradually from this day until 15 days postadministration for the dose of 4 mg of TD/kg bw. Finally, the ratio  $AUC_{\text{tonsil}}/AUC_{\text{plasma}}$  was 97.9, and the  $T_{1/2 (h)}$  was clearly higher in tonsil than in plasma.

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 Lorenzo Fraile, Departament de Producció Animal, ETSEA, University de Lleida, Lleida, Spain. E-mail: lorenzo.fraile@prodan.udl.cat

Tildipirosin (TD) is a semisynthetic tylosin analog, which has a unique chemical structure characterized by two piperidine substituents on C20 and C23, and a basic mycaminose sugar moiety at C5 of the macrocyclic lactone ring that has been approved for the treatment for respiratory diseases in pigs and cattle (EMA, 2011). This macrolide is rapidly absorbed and extensively distributed to the site of respiratory infection. In lung, mean TD concentrations were characterized by a peak on day 1 and a slow decline until 17 days after administration (Rose *et al.*, 2013). The long persistence of TD in the body is demonstrated further by a pronounced terminal elimination half-life of about 7 days in lung. As a class, the macrolides are characterized by extensive partitioning into tissues, where they achieve multifold higher concentrations relative to those observed in the blood plasma (Nightingale, 1997). The magnitude of the local accumulation and long persistence of TD in the lung results in a convenient treatment regimen (single administration) and positive clinical outcome rates for respiratory conditions (Rose *et al.*, 2013).

One critical point in the epidemiology of *Actinobacillus pleuropneumoniae* (APP) is that pigs can become asymptomatic

carriers of this micro-organism in their tonsils for long periods. In the literature, antimicrobial treatments have been used to eradicate APP from tonsils. Thus, Fittipaldi *et al.* (2005) used feed medicated with tilmicosin phosphate for 30 days but found that the tonsils of the majority of animals were APP PCR positive 30 days later. Other authors were also unable to eliminate this bacterium from tonsils (Angen *et al.*, 2008) with the usual administration schedule recommended for tulathromycin (2.5 mg/kg bw/one shot). Finally, an eradication program that includes sow medication with enrofloxacin (a fluoroquinolone) seemed to be successful, but specific studies to demonstrate the presence or not of APP in tonsils were not performed (Bækbo, 2006). Thus, the goal of this study was to quantify the TD penetration in tonsils and to characterize its pharmacokinetic profile at the registered dose (4 mg of TD/kg bw) as a first step to check the potential use of this molecule to eradicate APP from tonsils in carrier animals.

Forty-eight 2-month-old clinically healthy hybrid pigs (Landrace × Large white) were selected for this study. Animals were randomly divided into six groups (control, T1, T2 (1), T2 (5), T2(10), and T2(15)) of eight animals. T1 and T2 groups

received a dose of 2 and 4 mg of TD/kg bw in one shot, respectively, and the control group received 2 mL of saline solution (NaCl 0.9%). Depending on the pig weight, the administration volume of 40 mg of TD/mL (Zuprevo<sup>®</sup>; MSD Animal Health) ranged from 0.75 to 1 and 1.5 to 2 mL for the 2 and 4 mg dose of TD/kg bw, respectively. All the treatments were administered intramuscularly in the neck. Groups were balanced by gender and weight. The animals were sacrificed by intravenous administration of pentobarbital sodium 24 h after finishing the treatment for the control, T1, and T2(1) groups, whereas animals of T2(5), T2(10), and T2(15) were sacrificed at 5, 10, and 15 days, post-treatment, respectively. Tonsils and blood samples were taken at necropsy to quantify the concentration of TD (Intervet Innovation GmbH, a member of the MSD Animal Health group in Schwabenheim, Germany). Briefly, these samples were assayed for TD using high-performance liquid chromatography (HPLC) with a Spark HySphere<sup>®</sup> C18 HD cartridge (Spark Holland B.V.) with tandem mass spectroscopy detection (LC/MS/MS) after solid-phase extraction (Online-SPE). Details of the extraction method and phase mobile have been previously published (Rose *et al.*, 2013). Quantitation of tildipirosin was performed using a standard curve consisting of eight concentrations ranging from 0.025 to 10 µg/mL (plasma) and 0.125 to 50 µg/g (tonsil homogenate) of tildipirosin. The lower limit of quantification (LLOQ) was 0.025 µg/mL (plasma) and 0.125 µg/g for tonsils.

Plasma and tonsil PK parameters were determined based on group mean values with a noncompartmental analysis using the computer program Pharsight WinNonlin<sup>®</sup> software version 5.1.1 and 5.2.1 (Pharsight Corporation, St. Louis, MO, USA). Area under the curve (AUC) of plasma and tonsil concentration vs. time (AUC<sub>plasma</sub> and AUC<sub>tonsil</sub>) was measured via a linear trapezoidal method with linear interpolation. The start of the terminal elimination phase was set to 24 h after administration as previously published (Rose *et al.*, 2013).  $\lambda_z$  is defined as the first-order rate constant associated with the terminal (log-linear) portion of the curve, which is estimated by linear regression of time vs. logarithmic concentration. Finally, the terminal half-life ( $T_{1/2}$ ) was calculated as  $\log_{10}2/\lambda_z$ .

All statistical analyses were carried out using R software (R Core Team, 2012). In these analyses, the individual pig was the experimental unit. The significance level ( $\alpha$ ) was set at 0.05. Shapiro–Wilk's and Levene tests were used to evaluate the normality of the distribution of the variables and the homogeneity of variances, respectively. A nonparametric (Wilcoxon test) was chosen to compare the different TD concentration observed in plasma and tonsils between groups.

Average tildipirosin plasma (ATPC) and tonsil (ATTC) concentration for the control, T1, and T2(1) group increased significantly ( $P < 0.05$ ) in a dose-dependent manner, and the tonsil TD vs. plasma TD concentration ratio was approximately 75 at 24 h postadministration for the doses of 2 and 4 mg of TD/kg bw. On the other hand, the concentration in plasma was always significantly lower ( $P < 0.05$ ) than in tonsil for the groups treated with 4 mg of TD/kg bw at 1, 5, 10, and 15 days post-treatment, while the maximum concentration of

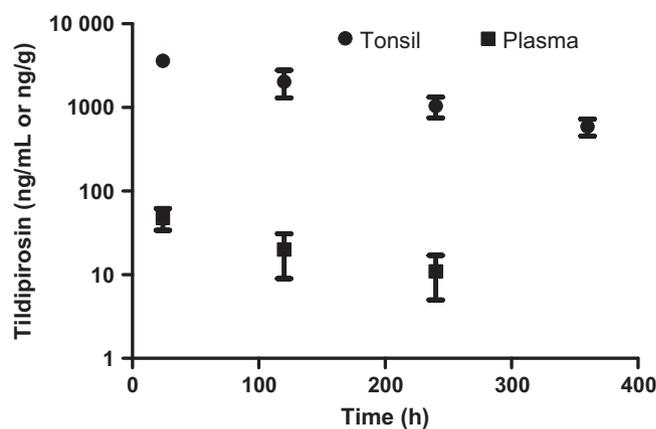


Fig. 1. Mean + SD tildipirosin concentration in porcine plasma and tonsil obtained postmortem at necropsy following a single i.m. administration at a dose of 4 mg of TD/kg bw.

tildipirosin in tonsil was observed at 1 day postadministration with a gradual decrease until 15 days postadministration (Fig. 1). Moreover, the ratio  $AUC_{\text{tonsil}}/AUC_{\text{plasma}}$  was 97.9, and the  $T_{1/2}$  (h) was clearly higher in tonsil than in plasma (Table 1).

Tildipirosin exerts a positive role in the treatment and control of bovine and swine respiratory disease (EMA, 2011). This clinical efficacy can be explained by the extraordinary lung selectivity and concentration vs. time profile of this macrolide in pulmonary tissue (Rose *et al.*, 2013). In the particular case to eliminate bacteria with antimicrobials from tonsils, the information generated in this study is necessary to know the pharmacokinetic profile of TD in tonsils because a similar pharmacokinetic profile cannot be assumed between airway compartments and tonsil. The concentration of tildipirosin in lung and bronchoalveolar lavage far exceeded plasma concentrations (Rose *et al.*, 2013) as it has been also described for tula-thromycin in pigs (Villarino *et al.*, 2013a,b). The basic nature of the drug and a limited degree of ionization at physiological pH

**Table 1.** Tildipirosin concentrations and its pharmacokinetic parameters in tonsil and blood plasma (mean+SD) and its ratio thereof after single i.m. administration of 40 mg/mL tildipirosin solution for injection at 4 mg of TD/kg bw

Time after administration (hours)	Tonsil concentration (ng/g)	Plasma concentration (ng/mL)	Ratio of tonsil vs. plasma
24	3620 ± 386.3	48 ± 14	75.4
120	2041.3 ± 740	20 ± 11	102
240	1043.9 ± 292	11 ± 6	95
360	590.1 ± 136	b.l.q	n.a.
Pharmacokinetic parameters (calculated from mean values)			
AUC <sub>last</sub> (h·µg/g or h·µg/mL)	837.796	8.556	97.9
$\lambda_z$ (/h)	0.00235	0.00335	0.70
$T_{1/2}$ (h)	295	207	1.43

b.l.q, Below limit of quantification; n.a. Not applicable.

represent features that favor the distribution of the drug into extravascular compartments (Cox *et al.*, 2010). The drug penetrated rapidly into the airways and tonsil, but concentration declined slowly. The drug concentration decay occurred faster in plasma than in the airways and tonsil. This result might indicate that different factors, other than/or in addition to simple drug diffusion, play a role in the process of movement of tildipirosin *in* and *out* between the plasma and the airway compartments and tonsils. The ratio TD tonsil vs. plasma concentration (75) is lower than the ratio TD lung vs. plasma concentration (83) at  $T_{max}$  (24 h postadministration) using a dose of 4 mg of TD/bw in one shot (Rose *et al.*, 2013). In contrast, the ratio  $AUC_{tonsil}/AUC_{lung}$  is 93.5% although the  $AUC_{tonsil}$  could be overestimated due to the lack of tildipirosin determinations before 24 h postadministration. In any case, this result suggests that the exposure of the lung to this drug (Rose *et al.*, 2013) is higher than the exposure at tonsil level.

Macrolides have been classified as time-dependent killing drugs, best described by the PK/PD parameter time above MIC ( $T > MIC$ ). However, for newer macrolides such as tulathromycin, gamithromycin, and tilmicosin, the plasma  $AUC/MIC$  ratio appears to be the best correlate with successful outcome (Evans, 2005; Lees *et al.*, 2006). In this case, the tildipirosin  $MIC_{90}$  for APP strains is 2  $\mu g/mL$  (Rose *et al.*, 2013). On the basis of tildipirosin concentration in tonsil and  $MIC_{90}$  for APP, the mean tonsil tildipirosin concentration is above the  $MIC_{90}$  for APP for about 5 days. However, this information is insufficient to predict whether the tonsil concentration is enough to eliminate this bacterium from this tissue and *in vivo* studies with carrier pigs are needed to confirm this assumption. The results from this study provide a good base to start working in this area.

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