

## Dose Dependent Penetration of Tulathromycin in Pig Tonsils

<sup>1</sup>Guillermo Cano, <sup>2</sup>Mark Hows, <sup>2</sup>Regina Janes, <sup>1</sup>Alberto Morillo and <sup>3,4</sup>Lorenzo Fraile

<sup>1</sup>Tests and Trials SL, Monzón, Spain,

<sup>2</sup>LGC limited, Cambridgeshire, United Kingdom,

<sup>3</sup>Department of Animal Production, ETSEA, University de Lleida, Lleida, Spain

<sup>4</sup>Agrotecnio Center, Lleida, Spain

### ABSTRACT

**Background and Objectives:** There is a paucity of information about the penetration of antimicrobials in tonsils from pigs with the final goal to eradicate bacteria from this tissue where different microorganisms can survive for long periods of time. The objective of the study was to quantify The Tulathromycin (TT) penetration in tonsils after applying several TT regimen dosages as a first step to check the potential use of this molecule to eradicate bacteria from tonsils. **Material and Methods:** Animals were randomly divided in four groups (control, T1, T2 and T3) of ten animals in each one. T1, T2 and T3 group received a dose of 2.5, 5 and 7.5 mg of TT kg<sup>-1</sup> body weight (bw) in one shot (Draxxin®), respectively and the control group received 2 mL of serum physiological. The animals were sacrificed by intravenous administration of pentobarbital sodium twenty four hours after finishing the treatment. Tonsils and blood samples were taken at necropsy to obtain serum. **Results:** The concentration in serum was always significantly lower ( $p < 0.05$ ) than in tonsil for the groups treated with TT. Average TT serum and tonsil concentrations increased significantly ( $p < 0.05$ ) in a dose-dependent fashion. Moreover, the tonsil TT versus serum TT concentration ratio was 5.4, 6.7 and 8.6 for the dose of 2.5, 5 and 7.5 mg kg<sup>-1</sup>, respectively. **Conclusions:** These results pave the way to use this antibiotic to eliminate bacteria from tonsils but additional studies are necessary to define correctly an administration schedule.

**Key words:** Macrolide, tonsil concentration, swine

Pharmacologia 6 (1): 45-51, 2015

### INTRODUCTION

Tulathromycin (TT) is a semi-synthetic macrolide antimicrobial. Its physicochemical feature allows the unionized fraction of the drug to easily penetrate into tissues from plasma and accumulate in compartments with acidic conditions<sup>1</sup>. In plasma, TT has a long terminal half-life and a remarkable large volume of distribution ( $V_d > 10 \text{ L kg}^{-1}$ ). Lung pharmacokinetic studies in pigs reveal an extraordinary capacity of TT for accumulation in lung tissue<sup>2,3</sup>. The magnitude of the local accumulation and long persistence of the drug in the target tissue (lung) results in a convenient treatment regimen (single administration) and positive clinical outcome rates for respiratory conditions<sup>4</sup>. TT, like other macrolides, is considered a bacteriostatic agent when tested against some microorganisms. However, the drug is bactericidal against *Actinobacillus pleuropneumoniae*

(APP) and *Pasteurella Multocida* (PM)<sup>5</sup>. The bactericidal action of macrolides has been described as time-dependent for erythromycin and concentration dependent for clarithromycin, azithromycin and tulathromycin for *Haemophilus somni*, particularly in the presence of plasma with post-antibiotic effect at least for APP<sup>6</sup>.

APP is the causative agent of porcine pleuropneumonia, a worldwide disease with occasional clinical outbreaks that can have a severe economic impact<sup>7</sup>. Attempts to control the disease have been made by vaccination, treatment with antibiotics and the establishment of herds free of the infection. Pigs can become asymptomatic carriers of the organism in their tonsils for long periods. Different antimicrobial treatments have been carried out to eradicate APP from tonsils. Thus, Fittipaldi *et al.*<sup>8</sup> used feed medicated with tilmicosin phosphate for 30 days but found that the tonsils of the majority of animals were PCR-positive 30 days later. Angen *et al.*<sup>9</sup> Also were unable to eliminate this bacterium from tonsils with the usual administration

Corresponding Author: Guillermo Cano, Tests and Trials SL, Monzón, Spain Tel: +34 973702814 Fax: +34 973702874

schedule recommended for TT (2.5 mg kg<sup>-1</sup> bw/one shot). Finally, an eradication program that includes sow medication with a fluorquinolone seemed to be successful but specific studies were not carried out to demonstrate the presence or not of APP in tonsils<sup>10</sup>.

To our knowledge, there is a paucity of information about the penetration of many antibiotics in tonsils including TT making difficult to draw clear conclusions about its potential efficacy to eliminate APP in carrier animals. Thus, the goal of this study was to quantify the TT penetration in tonsils after applying several TT regimen dosages as a first step to check the potential use of this molecule to eradicate this bacterium from tonsils.

## MATERIAL AND METHODS

**Experimental design:** Forty two-month old pigs weighting 12-18 kg were selected for this study coming from a farm with clinical cases of porcine pleuropneumonia. The APP strain was isolated from pneumonic lungs by Laboratories Ovis SL and its Minimal Inhibitory Concentration (MIC) was determined by LGC limited following broth microdilution methods published in VET01-A4<sup>11</sup>. The method was slightly modified by testing with 40% heat inactivated porcine serum and without an enriched atmosphere of 5% CO<sub>2</sub>. The pH of the MIC determination medium was maintained between 7.2 and 7.4. Animals were clinically healthy and have a good body condition at inclusion. All the pigs included in the study received non-medicated feed that was applied under commercial conditions in this farm. Animals were randomly divided in four groups (control, T1, T2 and T3) of ten animals. T1, T2 and T3 group received a dose of 2.5, 5 and 7.5 mg of TT kg<sup>-1</sup> of bw in one shot (Draxxin®), respectively and the control group received 2 mL of physiological serum. All the treatments were administered by intramuscular route in the neck. Groups were balanced by gender and weight. The animals were sacrificed by intravenous administration of pentobarbital sodium twenty four hours after finishing the treatment. Tonsils were taken and frozen at -80°C. At the same time, blood samples were taken at necropsy to obtain serum.

**Tulathromycin assay in serum and tonsil:** The concentration of TT in serum and tonsils was quantified by LGC limited. Briefly, these samples were assayed for tulathromycin concentration by a high-performance liquid chromatography with tandem mass spectrometry detection using chrysin as an internal standard<sup>12</sup>. To prepare standards, control serum and tonsils from animals which had not received any treatment were spiked with tulathromycin. Both methods were highly linear with coefficients of correlation of the standard

curves (r) better than 0.99. Accuracy and reproducibility were determined from inter-day and intra-day variances of assays with spiked concentrations. The limit of quantification was 5 ng mL<sup>-1</sup> and 30 ng gr<sup>-1</sup> for serum and tonsil, respectively.

**Statistical analysis:** All statistical analyses were carried out using R software (R Core Team; 2012). For all analyses, the individual pig was used as 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 non-parametric (Wilcoxon test) was chosen to compare the different TT concentration observed in serum and tonsils between groups.

## RESULTS

Average Tulathromycin Serum Concentration (ATSC) was 230+24.4, 427+50.5 and 554+45.6 ng mL<sup>-1</sup> for the T1, T2 and T3 group, respectively. Average Tulathromycin Tonsil Concentration (ATTC) was 1251+95.1, 2857+277.3 and 4751+376.3 ng gr<sup>-1</sup> for T1, T2 and T3 group, respectively. The concentration in serum was always significantly lower ( $p < 0.05$ ) than in tonsil for the groups treated with TT. ATSC and ATTC increased significantly ( $p < 0.05$ ) in a dose-dependent fashion with the exception of the serum concentration between the dose of 5 and 7.5 mg TT kg<sup>-1</sup> of bw (Fig. 1).

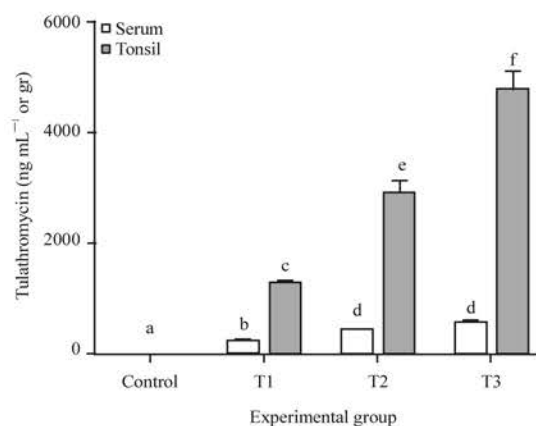


Fig. 1: Tulathromycin (TT) serum (white) and tonsil (black) mean concentration (ng mL<sup>-1</sup> or ng gr<sup>-1</sup>, respectively) after intramuscular application of saline physiological serum (control group) or TT at 2.5 (T1), 5 (T2) and 7.5 (T3) mg of TT kg<sup>-1</sup> of bw administered in one shot, Different letters between columns means statistically significant differences ( $p < 0.05$ )

Moreover, the tonsil TT versus serum TT concentration ratio was 5.4, 6.7 and 8.6 for the dose of 2.5, 5 and 7.5 mg kg<sup>-1</sup>, respectively. Finally, the MIC value for this particular strain was 4 μg mL<sup>-1</sup>.

## DISCUSSION

Several studies have shown that tulathromycin exerts a positive role in the treatment and control of bovine and swine respiratory disease<sup>13</sup>. An important reason for this is the extraordinary lung selectivity and concentration vs. time profile of this triamylide in pulmonary tissue<sup>2,3</sup>. In the particular case to eliminate bacteria with antimicrobials from tonsils, the target tissue is the tonsil. The main goal of this study was to quantify the penetration of tulathromycin in pig tonsils. It would have been ideal to define its pharmacokinetic tonsil profile using at least five samples times as it has been described for moxifloxacin in humans<sup>14</sup>. However, the quantification of this antibiotic in tonsil require to use the complete tonsil and consequently to sacrifice the animals. Thus, it was decided to use a representative number of animals (10) in one single sample time to minimize the number of animals used for welfare reasons. The sample time was chosen (24 h after intramuscular administration) taking into account the time to reach the maximum concentration (T<sub>max</sub>) observed in lung in previous studies<sup>2</sup>.

The concentration of tulathromycin in many tissues far exceeded plasma concentrations<sup>2,3</sup>. The basic nature of the drug and a limited degree of ionization at physiological pH represent features that favor the distribution of the drug into extra-vascular compartments<sup>4</sup>. The drug penetrated rapidly into the airways but concentration declined slowly. The drug concentration decay faster in plasma than in the airways. This suggests that there is not a rapid drug equilibration between both compartments after T<sub>max</sub>. This result might indicate that different factors other than/or in addition to simple drug diffusion, intervene in the process of movement of the tulathromycin in and out between the plasma and the airway compartments. In the case of tonsils, the ratio TT tonsil or lung versus plasma or serum concentration at T<sub>max</sub> is close to 6<sup>2</sup> for both tissues suggesting that its distribution is very similar in both cases.

Most macrolides have been classified as time-dependent killing drugs, best described by the PK/PD parameter time above MIC (T>MIC). However, AUC/MIC ratio has been proposed as the PK/PD variable that probably best predicts the antimicrobial activity of tulathromycin<sup>13</sup>. In this case, the MIC of the APP strain is 4 μg mL<sup>-1</sup> and it was not

feasible to calculate the AUC/MIC with this experimental design. Thus, if T>MIC is taking into account also as a suitable PK/PD parameter linked with clinical efficacy, only the highest dose tested (7.5 mg kg<sup>-1</sup>) will be able to achieve enough concentrations at tonsil level (4.7 μg mL<sup>-1</sup>) to eliminate this bacterium from the target tissue. In the literature, it has been previously published that the dose of 2.5 mg kg<sup>-1</sup> of bw was unable to eliminate this bacterium from tonsils in a study that did measure neither the antimicrobial concentration nor the MIC in the tonsil and strains present in the farms<sup>9</sup>. According to results of present study, the results obtained by Angen *et al.*<sup>9</sup> are easily understandable because the antimicrobial concentration obtained in tonsil (1.25 μg mL<sup>-1</sup>) is much lower than the MIC<sub>90</sub> value described in the literature of TT for APP (4 μg mL<sup>-1</sup>). Thus, as a speculation, it would have been necessary to use, at least, three times the dose registered for tulathromycin (7.5 mg kg<sup>-1</sup> of bw) if the goal is to eradicate the bacteria from tonsils. In any case, it must be highlighted that it is necessary to carry out additional studies to know the pharmacokinetics of TT in tonsil before defining correctly an administration schedule to maintain the concentration above MIC for enough period of time to eliminate this bacterium from tonsils.

## CONCLUSION

These results pave the way to use this antibiotic to eliminate bacteria from tonsils but additional studies are necessary to define correctly an administration schedule.

## ACKNOWLEDGEMENTS

The study was funded by Zoetis Spain S.L.U.

## REFERENCES

1. Norcia, L.J., A.M. Silvia, S.L. Santoro, J. Retsema and M.A. Letavic *et al.*, 2004. *In vitro* microbiological characterization of a novel azalide, two triamylides and an azalide ketal against bovine and porcine respiratory pathogens. *J. Antibiotics*, 57: 280-288.
2. Villarino, N., S. Lesman, A. Fielder, D. Garcia Tapia and S. Cox *et al.*, 2013. Pulmonary pharmacokinetics of tulathromycin in swine. Part I: Lung homogenate in healthy pigs and pigs challenged intratracheally with lipopolysaccharide of *Escherichia coli*. *J. Vet. Pharmacol. Ther.*, 36: 329-339.
3. Villarino, N., S. Lesman, A. Fielder, D. Garcia-Tapia and S. Cox *et al.*, 2013. Pulmonary pharmacokinetics of tulathromycin in swine. Part 2: Intra airways compartments. *J. Vet. Pharmacol. Ther.*, 36: 340-349.

4. Cox, S.R., C. McLaughlin, A.E. Fielder, M.F. Yancey and T.L. Bowersock *et al.*, 2010. Rapid and Prolonged distribution of tulathromycin into lung homogenate and pulmonary epithelial lining fluid of Holstein calves following a single subcutaneous administration of 2.5 mg/kg body weight. *Int. J. Applied Res. Vet. Med.*, 8: 129-137.
5. Godinho, K.S., S.G. Keane, I.A. Nanjiani, H.A. Benchaoui and S.J. Sunderland *et al.*, 2004. Minimum inhibitory concentrations of tulathromycin against respiratory bacterial pathogens isolated from clinical cases in European cattle and swine and variability arising from changes in *in vitro* methodology. *Vet. Ther.: Res. Applied Vet. Med.*, 6: 113-121.
6. Bair, U., H. Li, C. Wu, L. Zhou and X. Cao, 2010. *In vitro* activity and postantibiotic effect of tulathromycin against *Actinobacillus pleuropneumoniae*. *Anim. Husbandry Feed Sci.*, 2: 32-33.
7. Gottschalk, M. and D.J. Taylor, 2006. *Actinobacillus Pleuropneumoniae*. In: *Diseases of Swine*, Straw, B.E., J.J. Zimmerman, S. D'Allaire and D.J. Taylor (Eds.). 9th Edn., Blackwell Publishing, Ames, Iowa.
8. Fittipaldi, N., C. Klopfenstein, M. Gottschalk, A. Broes, M.A. Paradis and C.P. Dick, 2005. Assessment of the efficacy of tilmicosin phosphate to eliminate *Actinobacillus pleuropneumoniae* from carrier pigs. *Can. J. Vet. Res.*, 69: 146-150.
9. Angen, O., M. Andreasen, E.O. Nielsen, A. Stockmarr and P. Baekbo, 2008. Effect of tulathromycin on the carrier status of *Actinobacillus pleuropneumoniae* serotype 2 in the tonsils of pigs. *Vet. Rec.*, 163: 445-447.
10. Baekbo, P., 2006. Elimination of respiratory diseases-a Danish practitioners approach. *Proceedings of the 19th IPVS Congress of Copenhagen*, Volume 1, July 16-19, 2006, Denmark, pp: 313.
11. CLSI, 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Approved standard-fourth edition. CLSI Document VET01-A4, Clinical and Laboratory Standards Institute, Wayne, PA.
12. Galer, D., S. Hessong, B. Beato, J. Risk and P. Inskeep *et al.*, 2004. An analytical method for the analysis of tulathromycin, an equilibrating triamilide, in bovine and porcine plasma and lung. *J. Agric. Food Chem.*, 52: 2179-2191.
13. Evans, N.A., 2005. Tulathromycin: An overview of a new triamilide antibiotic for livestock respiratory disease. *Vet. Ther.*, 6: 83-95.
14. Esposito, S., S. Noviello, G. D'Errico, G. Motta and D. Passali *et al.*, 2006. Concentration of moxifloxacin in plasma and tonsillar tissue after multiple administration in adult patients. *J. Antimicrobial Chemotherapy*, 57: 789-792.