

## Pharmacokinetics and tissue depletion of tilmicosin in turkeys

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Tilmicosin is a macrolide antibiotic chemically synthesized from tylosin, with antimicrobial activity against Gram positive anaerobic bacteria, *Mycoplasma* spp., and Gram negative respiratory pathogens including *Mannheimia haemolytica* and *Pasteurella multocida*. Desirable pharmacokinetic properties of tilmicosin include rapid absorption following oral administration, good penetration of respiratory tract tissues and slow elimination. Tilmicosin is commercially available in Canada as Micotil® (Elanco Animal Health, Guelph, Ontario, CA), an injectable formulation approved for the treatment of respiratory disease in cattle and sheep and as Pulmotil® (Elanco Animal Health, Guelph, Ontario, CA), a feed pre-mix approved for the treatment and control of respiratory disease caused by *Actinobacillus pleuropneumoniae* and *P. multocida* in swine. In many countries outside of North America, tilmicosin is used for the treatment of respiratory tract infections in poultry caused by *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale* and *P. multocida* (Jordan & Horrocks, 1996; Kempf *et al.*, 1997; Jordan *et al.*, 1999; Abu-Basha *et al.*, 2007). Anecdotally, an outbreak of fowl cholera in Alberta turkeys caused by *P. multocida* appeared to be successfully treated by one of the authors (Inglis) when approved antimicrobials were ineffective. The objective of this study was to perform a pharmacokinetic analysis of oral tilmicosin in turkeys to predict therapeutic efficacy against *P. multocida*, a dosage regimen and a suitable withdrawal interval to prevent detectable residues.

This study was approved by the University of Saskatchewan's Committee on Animal Care and Supply in accordance with the guidelines provided by the Canadian Council on Animal Care. Seventy-four female 9 week old Hybrid Converter turkeys were obtained from a commercial farm in Saskatchewan. The birds were fed a standard turkey grower diet and water *ad libitum*. Five birds were initially euthanized to provide blank tissue

samples and the remaining 69 birds were placed on a diet containing 200 ppm tilmicosin and the birds were allowed to consume the medicated diet *ad libitum* for 5 days, then fed drug free turkey grower diet for the remainder of the study. Birds were euthanized in groups of five (four in the last group) after 3, 4 and 5 days on medicated feed (referred to as days -2, -1, 0), then 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 and 35 days after discontinuing treatment. Samples of lung, liver, kidney and breast muscle were collected from each bird at the time of slaughter. Tilmicosin concentrations in turkey tissues were determined using a high performance liquid chromatography (HPLC) method initially developed by the Canadian Food Inspection Agency (CFIA) for edible tissues (Chan *et al.*, 1994) and adapted in an elk study to measure lung concentrations (Clark *et al.*, 2004), (Table 1).

Pharmacokinetic analysis was performed using WinNonLin (ver2.1 Pharsight Corp., Raleigh, North Carolina, USA), employing a non-compartmental extravascular input model with uniform weighting. Values for the maximum concentration (C<sub>max</sub>) and time to maximum concentration (T<sub>max</sub>) were taken from the data. Tissue depletion parameters were calculated employing a non-compartmental extravascular input model with uniform weighting. Tissue concentrations from Day 0 (C<sub>Day0</sub>) were used to calculate  $\lambda$ , the terminal slope of the depletion curve. The goodness of fit of the models was estimated using the adjusted R<sup>2</sup> and all models had a R<sup>2</sup> of greater than 0.8.

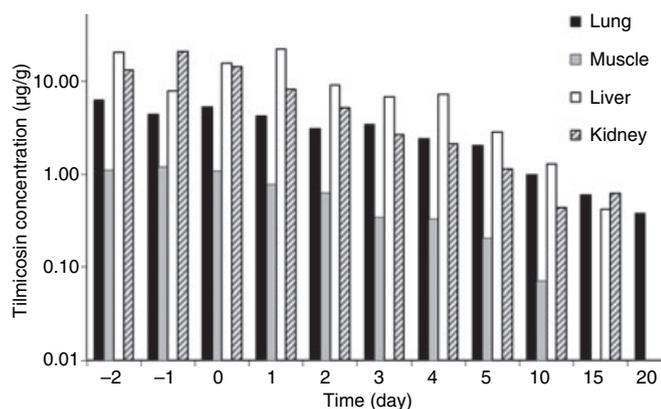
Validation of the HPLC assay for tilmicosin had been conducted in this laboratory for equine (unpublished data) and elk tissues (Clark *et al.*, 2004). The recovery, limits of detection and limits of quantification of the method are shown in Table 2. The minimum inhibitory concentration for tilmicosin was determined for 84 unclassified avian isolates of *P. multocida* from the veterinary microbiology laboratory collection (1987–

**Table 1.** HPLC parameters for tilmicosin assay

| Tissue | Tissue quantity (g) | Standard curve ( $\mu\text{g/g}$ ) | Tylosin internal standard ( $\mu\text{g/g}$ ) | Injection volume ( $\mu\text{L}$ ) |
|--------|---------------------|------------------------------------|---|------------------------------------|
| Liver  | 2.5                 | 0.3, 0.5, 1.0, 1.5                 | 4.0   | 20                                 |
| Lung   | 1                   | 0.5, 1.0, 2.0, 3.0                 | 10.0  | 20                                 |
| Muscle | 2.5                 | 0.25, 0.5, 1.0, 1.5                | 4.0   | 50                                 |
| Kidney | 1                   | 0.5, 1.0, 2.0, 3.0                 | 10.0  | 20                                 |

**Table 2.** Validation of the tilmicosin assay

| Tissue | Tilmicosin recovery (%) | Tylosin recovery (%) | Limit of detection ( $\mu\text{g/g}$ ) | Limit of quantitation ( $\mu\text{g/g}$ ) | Coefficient of variation (%) |
|--------|-------------------------|----------------------|--|---|------------------------------|
| Liver  | 95                      | 98                   | 0.163                                  | 0.543                                     | 5                            |
| Lung   | 90                      | 84                   | 0.211                                  | 0.704                                     | 16                           |
| Muscle | 83                      | 73                   | 0.059                                  | 0.197                                     | 6                            |
| Kidney | 89                      | 66                   | 0.178                                  | 0.590                                     | 4                            |

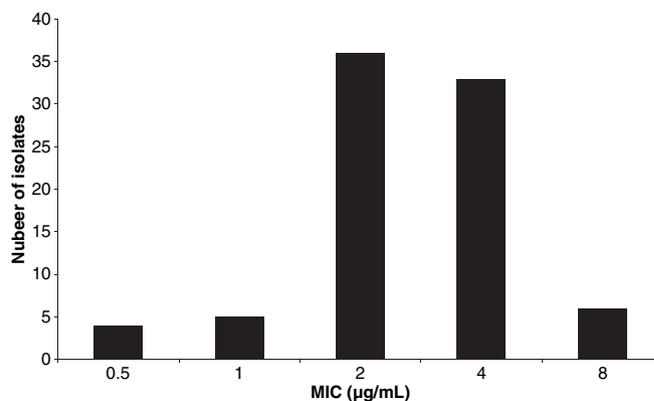
**Fig. 1.** Concentration (mean  $\pm$  SD) of tilmicosin in lung, muscle, liver and kidney from turkeys administered 200 ppm in feed for 5 days. Medicated feed was discontinued after Day 0.

2005) using the agar dilution method according to Clinical Laboratory Standards Institute (CLSI, formerly known as the National Committee for Clinical Laboratory Standards or NCCLS) guidelines. The swine premix formulation of tilmicosin in feed was well tolerated by the turkeys and no adverse reactions were noted. The mean tissue concentrations of tilmicosin vs. time are shown in Fig. 1 and the pharmacokinetic parameters are shown in Table 3. Day 0 represents the last day that the turkeys received medicated feed. Turkeys were sacrificed on Day -2 and -1 after being on treatment for 3 and 4 days, respectively. With oral dosing at 200 ppm for 5 days, the highest concentrations of tilmicosin were reached in liver, followed by kidney, lung, and muscle tissues. Tissue samples were analyzed until all samples from two subsequent time periods were below the limit of detection of the assay. According to CLSI guidelines, the susceptibility breakpoint for bovine and porcine isolates of *P. multocida* is a minimum inhibitory concentration (MIC) of  $\leq 8 \mu\text{g/mL}$ .

**Table 3.** Pharmacokinetic parameters of tilmicosin in turkey tissues

| Tissue | Adjusted $R^2$ of model | $C_{\text{max}}$ ( $\mu\text{g/g}$ ) | $T_{\text{max}}$ (day) | $C_{\text{Day 0}}$ ( $\mu\text{g/g}$ ) | $\lambda$ (/day) |
|--------|-------------------------|--------------------------------------|------------------------|--|------------------|
| Lung   | 0.98                    | 6.3                                  | -2                     | 5.39                                   | 0.132            |
| Muscle | 0.97                    | 1.2                                  | -1                     | 1.09                                   | 0.276            |
| Liver  | 0.88                    | 22.6                                 | 1                      | 16.02                                  | 0.28             |
| Kidney | 0.92                    | 20.9                                 | -2                     | 14.50                                  | 0.35             |

$C_{\text{max}}$ , the maximum tissue concentration;  $T_{\text{max}}$ , the day relative to the last day of medicated feed where the  $C_{\text{max}}$  was measured;  $C_{\text{Day 0}}$ , tissue concentration when medicated feed was discontinued;  $\lambda$ , terminal slope of the depletion curve.

**Fig. 2.** Distribution of minimum inhibitory concentrations of tilmicosin for 84 avian isolates of *Pasteurella multocida*.

The 84 isolates of avian *P. multocida* in our laboratory collection were a unimodal population with all MIC values  $\leq 8 \mu\text{g/mL}$  (Fig. 2).

Fowl cholera is a contagious, widely distributed disease caused by *P. multocida* that affects domestic and wild birds, but is most severe in turkeys. It usually occurs as a septicemia of sudden onset with high morbidity and mortality. In naturally occurring outbreaks, mortality ranges from 17–68%, while experimentally induced disease causes 90–100% mortality in exposed turkeys, with death usually occurring within a few days of infection (Carpenter *et al.*, 1988, 1989). Based on *in vitro* antimicrobial susceptibility, flock and farm history and the clinical judgment of the veterinarian, antimicrobial intervention is warranted due to the peracute nature of this disease. Macrolide antimicrobials are usually effective against *P. multocida* and tilmicosin appeared effective in controlling a fowl cholera outbreak in turkeys in Alberta.

The maximum concentration ( $C_{\text{max}}$ ) of tilmicosin in homogenized lung for turkeys ( $6.3 \mu\text{g/g}$ ) was higher than that reported for swine lung ( $1.43 \mu\text{g/g}$ ) when dosed at 200 ppm PO in feed, but was lower than that reported for chicken lung ( $7.96 \mu\text{g/g}$ ) when dosed at 50 mg/kg PO in water and cattle lung ( $7.17 \mu\text{g/g}$ ) when dosed at 10 mg/kg SC (FDA, 1992, 1996; Keles *et al.*, 2001). In all of these species, tilmicosin concentrations are minimally detectable in serum and homogenized lung concentrations are below  $8 \mu\text{g/g}$  but nevertheless

tilmicosin is considered highly efficacious in the treatment of respiratory disease. This clinical efficacy despite sub-MIC serum and lung concentrations has been attributed to three mechanisms. First, tilmicosin accumulates within avian, porcine and bovine phagocytic cells, reaching high ratios of intracellular to extracellular drug concentration (Scorneaux & Shryock, 1999). In chickens, tilmicosin accumulates in heterophils and monocyte-macrophages. In addition, phagocytosis of *P. multocida* and lipopolysaccharide exposure increases tilmicosin uptake by the avian phagocytes and the presence of opsonized *P. multocida* enhances the release of tilmicosin from the phagocytes (Scorneaux & Shryock, 1998). In addition, intracellular tilmicosin increases cellular lysosomal production in all three chicken phagocyte types (Scorneaux & Shryock, 1998). Therefore, it is suggested that these intracellular concentrations contribute to tilmicosin's efficacy in respiratory tract infections in a way not predicted by serum or lung homogenate concentrations. Third, tilmicosin has a strong post antibiotic effect and post antibiotic sub-MIC effect on *P. multocida*, which may impede disease progression by allowing the animal's immune response to successfully eliminate a weakened bacterial population (Diarra *et al.*, 1999; Lim & Yun, 2001).

Peak tilmicosin concentrations and depletion from liver, kidney and muscle in turkeys were similar to that reported in chickens administered tilmicosin in water at 75 mg/mL (Zhang *et al.*, 2004) and 50 mg/mL (Keles *et al.*, 2001). The limits of detection of the assay for turkey tissues are higher than those reported for other HPLC techniques for tilmicosin in mammalian tissues (Chan *et al.*, 1994). This is due to the small size of the turkey kidneys and lung tissues, so 1 g aliquots of tissue were the maximum amounts that could be analyzed in the assay. Reducing the tissue sample size increased the probability of weighing errors that contributed to the higher detection limits.

Based on the results of this study, a dose of 200 ppm of tilmicosin premix in feed for 5 days should provide therapeutic concentrations for the treatment of fowl cholera in turkeys caused by *P. multocida*. Responsible extralabel drug use requires veterinarians to follow appropriate drug withdrawal times to prevent detectable drug residues at slaughter. Tilmicosin is approved in the European Union (EU) as an aqueous formulation for chickens and turkeys and the European Agency for the Evaluation of Medicinal Products (EMA) has set the tilmicosin MRLs at 1.0 µg/g in liver, 0.25 µg/g in kidney and 0.075 µg/g in muscle (EMA CVMP, 2000). Since tilmicosin is not approved for use in turkeys in Canada, there are no legal maximum residue levels (MRL) in turkey tissues. Therefore, any tilmicosin residue detected at slaughter by the Canadian Food Inspection Agency (CFIA) would be considered violative and would have serious consequences for the producer and prescribing veterinarian. The data from this study conducted in healthy turkeys predicts that tilmicosin residues in tissues would fall below EU MRLs by 20 days after a 5 day course of treatment, and by 25 days would be below the limits of detection for the methodology of this study, which is based on

CFIA methodology. Further studies are needed to confirm the clinical efficacy and residue depletion of tilmicosin in diseased turkeys.

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