Influence of anticoccidial agents on pharmacokinetics and tissue residues of tilmicosin and spiramycin in healthy and experimentally infected broiler chickens with coccidia

A Thesis Presented By

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INTRODUCTION

Pharmacokinetic drug interactions are of great clinical significance in veterinary practice. Drugs have the same metabolic pathway usually show drug interactions in its concomitant administration (Abdel Salam et al., 1986, Dalvi et al., 1987). These constitute a major problem facing the poultry production as many compounds have been usually add to poultry rations as feed additives (Jones and Ricke, 2003) which may interact with any antibacterial drugs used for treatment of bacterial diseases, affecting its kinetic behaviour. From this viewpoint dosages of antibacterial drugs used in poultry farms must be adjusted to compensate for its interaction with these feed additives.

Anticoccidials are one of these compounds, their programs have been substantially varied in its clinical application in poultry farms (Magee, 1992 and Echman, 1997).

Macrolides belong to the family of macrocyclic antibiotics. They form a homogeneous class of antibiotics in terms of their chemical structure (macrocyclic lactone nucleus). They have a wide range of antibacterial spectrum. Residues of these antibiotics in edible products may lead to allergic reactions in consumers and to the development of resistant bacteria.

Tilmicosin is a broad-spectrum bacteriostatic macrolide antibiotic synthesized from tylosin for veterinary use only. It has an antibacterial spectrum that is predominantly effective against Mycoplasma spp., Pasteurella spp. and various Gram-positive organisms (Ose, 1987; Prescott, 2000). It has been used extensively to treat respiratory disease in swine, cattle and sheep (Moore, 1996; Hoar et al., 1998; Christodoulopoulos et al., 2002). Tilmicosin is licensed for the treatment
and control of respiratory diseases associated with *Mycoplasma
gallisepticum*, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale*
and *Pasteurella multocida* in broiler chickens (Jordan and Horrocks,
1996; Kempf et al., 1997; EMEA, 1998; Varga et al., 2001; Jordan et
al., 1999; Abu-Basha et al., 2007). Tilmicosin has a stronger antimicrobial
activity than tylosin against *Pasteurella haemolyticus*, *Pasteurella
multocida*, and *Mycoplasma* (Stephens et al., 1993, Inamoto and
Kikuchik, 1994).

Spiramycin is a macrolide antibiotic; It has been isolated by
Pinnert-Sindico et al., 1954 from cultures of *Streptomyces ambofaciens*.
It is active against gram-positive organisms, some gram-negative bacteria
and other organisms, including *Mycoplasma pneumoniae*, *Chlamydia
trachomatis*, *Toxoplasma gondii*, *Legionella pneumophila*, and
*spirrochetes* and also used as a growth promoter.

Toltrazuril is a symmetrical triazinetrione compound and 2.5% oral
solution has been shown to be effective against all species of *Eimeria*
infesting chickens (Mehlhorn et al., 1988). It is active against all
intracellular developmental stages including those of schizogony and
gametogony (Mehlhorn et al., 1984). Toltrazuril has chemoprophylactic
(Gjerde and Helle 1991) and therapeutic effects (Chapman, 1987;
Mehlhorn et al., 1988; Mathis et al., 2004; Ghanem et al., 2008)
against coccidiosis and does not interfere with the development of
immunity (Grief, 2000). Chemoprophylaxis with Toltrazuril enhances
immunity development (Grief, 2000).

Salinomycin is polyether antibiotic belonging to the group of
ionophores (KINASHI et al., 1973), This natural toxin is produced by a
*Streptomyces albus* strain (Miyazaki et al., 1974). Salinomycin is
extensively used as a coccidiostat in poultry and other livestock and is
commonly fed to ruminant animals to improve feed efficiency (Callaway et al., 2003, Yvoré et al., 1980) used extensively in the prevention of coccidiosis in broilers.

The purpose of this work to determine the effect toltrazuril and salinomycin (anticoccidial drugs) on the kinetic parameters and tissue residues of tilmicosin and spiramycin (macrolide antibiotics) in broiler chickens infected with coccidia.
REVIEW OF LITERATURE

In the present work, the effect of two anticoccidial drugs (toltrazuril and salinomycin) on the disposition kinetic and tissue residue of two macrolide antibiotics (tilmicosin and spiramycin, respectively) were studied in broiler chickens.

Macrolide antibiotics

Macrolides have a wide range of antibacterial spectrum. Residues of these antibiotics in edible products may lead to allergic reactions in consumers and to the development of resistant bacteria.

Tilmicosin:

Tilmicosin is a semisynthetic macrolide antibiotic used for 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 and Abu-Basha et al., 2007).

Gorham et al., (1990) evaluated the Tilmicosin in eight field trials as a single subcutaneous injection at dosages of 0, 5, 10 and 20 mg/kg for the treatment of naturally occurring respiratory disease in Feed lot cattle. At the time clinical signs of respiratory disease and body temperature of 40.6°C or higher were observed. Treated animals were evaluated daily for 10 days and finally at day 28. Each animal was weighed on the first day and again on day 28. All treatment dosages were effective in significantly lowering mortality, improving weight gains, lowering body temperature, and reducing the severity of clinical signs. Body temperature was the only variable with statistically significant differences among the dose levels.

Jordan et al., 1993; Main et al., 1996; Papich and Rieviere, 2001 mentioned that cardiovascular toxicity and deaths have been
reported when tilmicosin was administered as an intravenous bolus (i.v.) bolus or at doses much greater than therapeutic dose.

Administering veterinary drugs to animals without an appropriate withdrawal period may lead to violative residues in tissues. Tilmicosin residues in animal tissues have been determined by liquid chromatographic (LC) methods (Richard and Raj, 1994; Wayne et al., 1994), liquid chromatography-mass spectrometry (Delepine et al., 1996), and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (Kiehl and Kennington, 1995).

ZIV et al. (1995) studied Tilmicosin antibacterial activity and pharmacokinetics in cows. The minimal inhibitory concentration (MIC) of tilmicosin for 90% of 112 Staphylococcus aureus isolates from the bovine udder was 0.78 μg/ml and 149 of 164 (90.8%) other gram-positive udder pathogens were inhibited by tilmicosin concentrations < 3.12 μg/ml. The MIC of the drug for 19 of 22 S. aureus isolates was < 0.78 μg/mL when the test was conducted using Mueller-Hinton (MH) agar or MH agar containing 7.5% skimmed milk. Acute cardiac toxicity followed intravenous (i.v.) injection of the drug at 10 mg/kg to 3 cows, but animals appeared clinically normal within 30 min after treatment. The pharmacokinetics of i.v. administered tilmicosin is typical for the macrolide class of antibiotics, i.e. low serum drug concentrations and a large volume of distribution (> 2.0 L/kg). The elimination half-life (t_{1/2β}) values for 3 cows were 46.4, 56.0 and 72.8 min. The drug was administered subcutaneously (s.c.) to 5 cows at 10 mg/kg; the elimination half-life (t_{1/2el}) was 4.18 ± 0.55 h and the mean s.c. bioavailability was 22%. Rapid and extensive penetration of tilmicosin from blood into milk, and slow elimination from the milk were among the characteristic kinetic features of the drug after i.v. and s.c. administration. Tilmicosin was
injected s.c. at 10 mg/kg once to 9 cows after the last milking of lactation; dry udder secretion samples were collected daily for 11 consecutive days and assayed microbiologically. The Concentrations of drug > 0.78 μg/mL were found in the secretion for 8–9 days after dosing. Systemic side-effects were not observed after s.c. drug administration.

**Jordan and Horrocks, (1996)** found that the experimentally infected broiler chickens with *Mycoplasma synoviae* were successfully controlled tilmicosin administration in the drinking water. The kinetic studies have showed that tilmicosin is distributed in to lungs and air sacs in a concentration (2.3 ± 0.72 μg/g lung after 48 h of oral treatment) that exceeds the MIC for *Mycoplasma gallisepticum* (0.0125 μg/ml) and *Mycoplasma synoviae* (0.1 μg/ml).

**EMEA, European Union, (1996)** and the veterinary drug residue regulations of the Chinese Ministry of Agriculture, (2002) reported that the maximum residue levels (MRLs) of tilmicosin in broiler chicken muscle, liver, and kidney are 0.075, 1.0, and 0.25 μg/g, respectively.

**Kempf et al., (1997)** evaluated the effect of 5 day (in water) tilmicosin medication for prevention of experimental mycoplasma gallisepticum disease in a 10 day old SPF chickens. The birds were inoculated intra tracheally and in to the sinus with *mycoplasma gallisepticum* R-P10 strain and given tilmicosin from 8-13 days of age then observed for 11 days post challenge, the results showed that tilmicosin decreases growth losses and respiratory signs and reduced air sac lesions from *M. gallisepticum*.  
Ramadan, (1997) administered tilmicosin to goats intravenously and subcutaneously to determine its concentration in blood and milk and its kinetic behaviour. After a slow intravenous injection, the serum concentration-time curve indicated a two compartment open model with a mean (SEM) elimination half-life ($T_{1/2\beta}$ s) of 4.36 (0.04) hours. After a subcutaneous injection the drug was eliminated more slowly from serum and milk, with $T_{1/2\beta}$ s 29.3 and 41.4 hours, respectively. The apparent volume of distribution of tilmicosin was more than 1l/kg. The peak serum tilmicosin concentration was 1.56 µg/ml at 6.39 hours after a subcutaneous injection of 10 mg/kg. Tilmicosin was extensively secreted into milk, reaching a maximum concentration of 11.6 µg/ml and having a large AUC milk /AUC serum ratio of approximately 12:1. Tilmicosin was detectable in milk for 11 days after a single subcutaneous dose.

Warren et al., (1997) determined lung tissue and air sac concentrations of tilmicosin during administration over 3 days in drinking water indicated that tilmicosin was detected in lung and air sac tissues within 6 hours of its being offered in drinking water, and after 24 hours, the concentration in the air sac exceeded that in the lung. After the drug had been administered at a level of 75 mg/kg in drinking water for 3 days, the concentration in lung and air sac tissues were higher than that in muscle and lower than those in liver and kidney. After 2 days of withdrawal, the concentration in lung was higher than that in muscle and lower than those in liver and kidney, and the concentration in air sac was higher than those in muscle and kidney but lower that in liver. These results showed that air sac is another target tissue for tilmicosin residues in broiler chickens besides the liver.

Modric et al., (1998) investigated the pharmacokinetics and pharmacodynamics of tilmicosin in sheep and cattle. Serum
pharmacokinetics of tilmicosin were compared between cattle (major spp.) and sheep (minor spp.) after subcutaneous injection in order to evaluate a new potential application of the drug in currently non approved spp. There were no significant differences in the elimination rates, maximum serum concentrations, half-lives, areas under curve, areas under the first moment curve and mean residence time. Volume of distribution and clearance, when normalized by body weight, were also similar. The only significant different parameter was T\textsubscript{max}, with sheep having the T\textsubscript{max} of 3.9 h, compared to 0.5 h in cattle. Although macrolides are considered to be one of the safest anti-infective drugs, adverse cardiovascular effects of several macrolides have been reported. The cardiopulmonary effects of tilmicosin were monitored in healthy adult sheep after subcutaneous injection at the dose of 10 mg/kg, and no significant changes were found.

Atef et al., (1999) investigated the elimination of tilmicosin in lactating ewes after subcutaneous injection at a dose of 10 mg/kg b.wt. and determined its plasma, milk, urine and ruminal juice concentrations. Tilmicosin could be detected in all those fluids 30 min. after injection and its concentrations in milk and urine were higher than those of plasma and ruminal juice. C\textsubscript{max} in plasma and milk were 1.29 and 9.5 µg/ml and were obtained at T\textsubscript{max} 5.235 and 15.093 h., respectively. The drug was slowly eliminated from plasma and milk indicating its long T\textsubscript{1/2β} of 15.4 and 26.4 h., respectively.

Gaugain and Anger, (1999) used a HPLC method for determination of tilmicosin, tylosin, spiramycin, and its major metabolite neospiramycin, that is suitable for porcine, bovine and poultry muscle. Macrolides residues were extracted from muscle with acetonitrile, fat was removed by liquid-liquid extraction with isoctane, and the extraction
was then cleaned on Bond Elute C18 cartridges. The HPLC separation was performed on an inertsil ODS3 C18 column (150×4 mm) with 0.05% trifluoroacetic acid – acetonitrile in a gradient mode and the detection wave lengths were 287 and 232 nm for tilmicosin-tylosin and spiramycin-neospiramycin, respectively. The method was validated from 1/2 the maximum residue level (MRL) to 4 times the MRL with pork muscle samples. Mean recoveries were 60, 63.5, 51 and 42 % for tilmicosin, tylosin, spiramycin and neospiramycin, respectively. LOD were 15 µg/kg for tilmicosin and tylosin, 30 µg/kg for spiramycin and 25 µg/kg for neospiramycin.

De Rosa et al., (2000) tested bacterial isolates obtained from swine with various clinical diseases for susceptibility to tilmicosin by minimum inhibitory concentration (MIC) and Kirby-Bauer disk diffusion tests using National Committee on Clinical Laboratory Standards methodology. The tilmicosin MIC90 was ≤ 0.125 µg/ml for Erysiopelothrix rhusiopathiae, ≤ 1 µg/ml for Haemophilus parasuis isolates, 8 µg/ml for Actinobacillus suis and Pasteurella multocida type A, 16 µg/ml for toxigenic and non toxigenic P. multocida type D, 64 µg/ml for Bordetella bronchiseptica, and > 128 µg/ml for Staphylococcus hyicus and Streptococcus suis. The results of disk diffusion testing matched well with the MIC results for each pathogen. This in vitro survey of tilmicosin activity against various swine isolates suggests that further clinical evaluation of tilmicosin in swine may be warranted for disease associated with E. rhusiopathiae, H. parasuis, and A. suis but not B. bronchiseptica, S. suis, or S. hyicus.

EMEA CVMP, (2000) found that a dose of 20 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 extra label 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. The European Agency for the evaluation of Medicinal Products (EMEA) 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.

**Stobba-Wiley et al., (2000)** developed and validated a method was developed and validated for determination and quantitation of tilmicosin residues in swine, cattle, and sheep edible tissues, as well as chicken fat, skin, and muscle over a concentration range of 0.025 µg/g – 20 µg/g. For chicken kidney and liver, the method was validated over a range of 0.060 µg/g–20 µg/g. The tissue sample was extracted with methanol and a C18 cartridge was used for solid-phase extraction cleanup. A reversed phase gradient liquid chromatographic method with detection at 280 nm was used to separate the tilmicosin from matrix components in 30 min run time. The limit of quantitation (LOQ) of the method was 0.025 µg/g for all tested tissues except chicken kidney and liver, for which the LOQ was 0.06 µg/g. Average recoveries for tissue samples ranged from 73 to 98%. Relative standard deviation values ranged from 0.6 to 14.7%.

**Keles et al., (2001)** investigated the pharmacokinetics and tissue concentration of tilmicosin after oral administration of a single oral dose (50 mg/kg b.wt.) in fowl. After oral administration, tilmicosin pharmacokinetics conformed to a two compartment open model. Tilmicosin was slowly eliminated from the serum and lung with mean half-lives of 30.18 ± 2.38 and 75.74 ± 3.67 hours, respectively. The mean maximum concentration of the drug in the lungs was found to be 6.2 times greater that of serum. Serum and lung tilmicosin concentrations reached peak values 4.66 ± 2 and 17.78 ± 7.51 hours, respectively, after oral administration. In fowls, the apparent volume of distribution was
found to be more than 1L/kg, indicating extensive tissue distribution. The clearance values were calculated to be 1.33 ± 0.06 for serum and 0.11 ± 0.004 L/h for lung. After oral administration, tilmicosin appeared to be retained at higher concentrations and for longer times in the edible tissue than in serum. The mean peak concentrations of tilmicosin were obtained in kidney 8 hours after dosing and in liver, heart and muscles 12 hours after dosing. Then, tissue concentrations decreased slowly over time and tilmicosin residues were only detected in the liver and kidney.

**Moredo et al., (2001)** Investigated the pharmacokinetics and cardiac toxicity of tilmicosin after i.v. administration in pigs. They found that tilmicosin administered to swine as an i.v. bolus at a dose of 4.5 mg/kg caused adverse cardiovascular and death.

**Naccari et al., (2001)** studied the effectiveness and kinetic behavior of tilmicosin in the treatment of respiratory infections in sheep, suffering from all respiratory signs, received a single subcutaneous dose of 10 mg/kg b.wt. of tilmicosin. The clinical signs were eliminated within 4-6 days. More of the drug was absorbed by the infected animals and its concentration remained higher for significantly longer.

**Fajt et al., (2003)** studied the effects of danofloxacin and tilmicosin on neutrophil function and lung consolidation in beef heifer calves with induced *P. haemolytica* pneumonia and found that danofloxacin and tilmicosin have no clinically significant effects on neutrophil function or apoptosis.

**Gang et al., (2004)** studied the pharmacokinetics of tilmicosin at a single dose in chickens of Blood Stagnation Syndrome at a single oral dose of 20 mg/kg b. wt., the drug concentration in plasma was done in microorganism-test way. Calculation of kinetic parameters was performed using 3-P87-PK program. The results indicated: The main
PK-parameters of the healthy group were $t_{1/2a}$ (0.662 ± 0.354) h, $T_{1/2\beta}$ (36.344 ± 15.204), $K_{1/2a}$ (0.340 ± 0.132) h, $V/F$ (7.049 ± 4.320) L/kg, $C_{\text{max}}$ (1.108 ± 0.523) mg/L, $T_{\text{max}}$ (0.765 ± 0.163) h, CL (0.571 ± 0.138) L·h/kg, AUC (56.220 ± 31.933) mg·h/L and $t_{1/2a}$ (3.655 ± 2.470) h, $t_{1/2\beta}$ (45.576 ± 14.201) h, $K_{1/2a}$ (0.142 ± 0.101) h, $V/F$ (20.246 ± 9.416) L/kg, $C_{\text{max}}$ (1.405 ± 0.332) mg/L, $T_p$ (1.011 ± 0.534) h, CL (0.484 ± 0.113) L·h/kg, AUC (59.250 ± 36.160) mg·h/L, respectively.

*Zhang et al.*, (2004) modified and validated a high performance liquid chromatography method with detection at 290 nm for determination of tilmicosin residues in broiler chickens tissues. The limit of detection (LOD) 0.01 µg/g for muscle and 0.025 µg/g for liver and kidney. Average recoveries ranged from 80.4 to 88.3%. Relative standard deviation values ranged from 5.2 to 12.1%. Residue depletion study was examined after dosing over a 5 days period via drinking water at 37.5 and 75.0 mg/ml. Tilmicosin concentration in liver and kidney were highest on day 3 of medication and on day 5 in muscle, in both low and high dose groups. The residue levels in both groups were significantly higher in liver than in kidney or muscle. the lung is another target tissue for tilmicosin residues in broiler chickens besides the liver. A minimum withdrawal time of 9 days was indicated for residue levels in muscle, liver and kidney tissues below MRL.

*Shen et al.*, (2005) determined the pharmacokinetics of tilmicosin after oral administration of a single dose of tilmicosin in swine at a single dose of 20 mg/kg or 40 mg/kg. Blood samples were obtained from a jugular vein immediately before and at 10, 20, and 30 minutes and 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 hours after administration of tilmicosin. Tilmicosin concentrations in serum were quantified by use of a HPLC-UV. Tilmicosin concentrations in serum decreased in a bi
exponential manner after oral administration. Absorption half-lives were 1.49 ± 0.23 hours and 1.64 ± 0.40 hours, distribution half-lives were 2.96 ± 0.58 hours and 3.20 ± 0.76 hours, elimination half-lives were 25.26 ± 8.25 and 20.69 ± 5.07 hours, peak concentrations were 1.19 ± 0.30 µg/mL and 2.03 ± 0.28 µg/mL, and time to peak concentrations was 3.12 ± 0.50 hours and 3.48 ± 0.77 hours after oral administration of tilmicosin base at a single dose of 20 or 40 mg/kg, respectively. Finally tilmicosin was rapidly absorbed and slowly eliminated after oral administration of a single dose of tilmicosin base powder.

Dergham et al., (2006) recorded that, using tilmicosin (Provitil powder®) oral formulation (30 mg/kg b. wt. for 3 days every 5 weeks for 4 months) is considered a highly effective therapeutic in *Mycoplasma gallisepticum* control programs for broiler breeder chicken flocks, and has a net positive effect for the producer.

Haiyang jiang et al., (2006) studied the tissue residue depletion of tilmicosin in eighteen cattle after a single subcutaneous injection of therapeutic level of 10 mg/kg b.wt. Three treated animals were slaughtered at 1, 7, 14, 28 and 35 days withdrawal after injection. Samples of the injection sites and of muscle, liver, kidney and fat were collected. Tilmicosin concentrations were determined using HPLC- UV at 290 nm. Using a statistical method recommended by the EMEA, the withdrawal time of 34 days was established when all tissue residues except sample in the injection sites were below the accepted MRL.

Abu-Basha et al., (2007) studied the bioavailability and pharmacokinetics study of powder and liquid tilmicosin formulations was carried out in 18 healthy chickens according to a single-dose, two-period, two-sequence and crossover randomized design. The two formulations were Provitil and Pulmotil AC. Both drugs were administered to each