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# Pharmacokinetics of levamisole in broiler breeder chickens

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The pharmacokinetics of levamisole was studied in 20 broiler breeder chickens (chickens that give eggs to breed broilers). A single dose of levamisole (40 mg/kg) was administered orally or intravenously to chickens before the onset of egg production, prelay (age = 22 weeks), and repeated at the peak of egg production (age = 32 weeks). A high-pressure liquid chromatographic with ultraviolet detection method (HPLC-UV) was used for quantification of levamisole in plasma. Using compartmental analysis, levamisole followed a three-compartmental open model with mean values of $\alpha = 0.1224$ and 0.4968, $\beta = 0.01663$ and 0.01813, $\gamma = 0.002$ and 0.002/min at the prelay and at the peak of egg production periods, respectively. The mean values for volume of distribution at steady state ($V_{ss}$), determined by compartmental analysis, were significantly different for prelay and peak of egg production (8.358 and 13.581 mL/kg), respectively.

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## INTRODUCTION

Levamisole is the laevo isomer of di-tetramisole, which is a racemic mixture. The parent compound tetramisole was first marketed as an anthelmintic in 1965 but it was soon noted that its anthelmintic activity resided almost entirely in the L-isomer, levamisole. Thus, it was determined that by using the L-isomer alone the dosage could be reduced by half. Reducing the dosage has an advantage of decreasing the risk of toxicity with the same anthelmintic potency (Barragry, 1994).

Levamisole is widely used as anthelmintic in cattle, sheep, goats, swine, and poultry. It is effective against lungworms and gastrointestinal nematodes. It is also used as adjuvant therapy in the treatment of human cancer and it is not approved by the FDA for use in poultry (US FDA, 2004), because of limited data regarding pharmacokinetics and withdrawal time; however, it is used for treatment of capillariasis in chickens (USDA, Food saftey Inspection Services, 1998).

Nematodes constitute the most important group of helminthes that infest poultry in both number of species and extent of damage they cause, far exceeding trematodes and cestodes (Ruff & Norton, 1997). Adult worms are commonly diagnosed by necropsy of broiler flocks, ages 4–9 weeks, and in breeder pullets and males, ages 4–25 weeks, and in adult heavy breeder laying hens (Dawe & Hofacre, 2002).

The purpose of the present work was to study pharmacokinetic parameters for levamisole in chickens, a target species for this compound. The pharmacokinetics was determined in chickens prior to egg laying and at peak egg laying to determine if egg laying would affect the optimum levamisole dose for chickens. The dose of levamisole used (40 mg/kg) was chosen according to Pankavich *et al.* (1973); Manger (1991); Charles and Roberson (2001) and Ruff and Norton (1997) because this dose effectively killed above 95% adult forms of *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria obsignata* and eliminated a high percentage of the larval stages of these parasites.

## MATERIALS AND METHODS

### *Chickens and experimental design*

The experiment was carried out with 20 clinically healthy female broiler breeder chickens that were kept in wire cages at Auburn University Poultry Science Farm, Auburn, AL, USA. At age 19 weeks, they were started on a drug free standard broiler breeder ration. At age 22 weeks (prelay), chickens were randomly placed into two groups ($n = 10$). One group was given a single oral dose (solution) and the other a single i.v. injection into the left wing vein of levamisole HCl solution 40 mg/kg. At age 32 weeks (at the peak of egg production), the chickens that had received the single oral or i.v. dose were again given a single oral or i.v. dose. Since the primary objective of this investigation was to ascertain the influence of egg production on levamisole pharmacokinetics, only one factor, egg production, was allowed to vary within each bird. Due to time restrains of egg laying stages a parallel design was used for comparing the routes of administration and it was not possible to conduct both i.v. and oral studies within the same chickens during that particular stage.

*Sampling*

Blood samples (2 mL each) were collected from all birds by serial puncture of the right wing vein in heparinized tubes, with heparin sodium salt, at 0, 7, 15, 30 min, 1, 2, 4, 6, 8, 12, 18, 24 and 30 h after i.v. injection and at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24 and 30 h after oral administration of levamisole HCl. The blood samples were centrifuged for 5 min at 848 × ***g*** and plasma was separated. Plasma samples were stored at −20°C until analyzed. The total amount of blood taken from each bird was 22 mL/day (0.48% of the total body weight). Birds can survive blood loss up to 10% of its body weight (Kovach *et al.*, 1969).

*Analytical procedures*

Calculation of levamisole in plasma was quantified by a high-pressure liquid chromatographic with ultraviolet detection (HPLC-UV) method according to El-Kholy and Kemppainen (2003). A polypropylene centrifuge tube (15 mL) containing 1 mL plasma was spiked with 100 μL of methyllevamisole at concentration of 10 μg/mL as an internal standard and 0.9 mL water was added and vortex mixed, 0.5 mL of 10 N sodium hydroxide added, vortex mixed, 5 mL of ethyl ether:n-hexane (80:20, v/v) added and vigorously shaken. The mixture was centrifuged for 5 min at 848 × ***g*** and the organic layer was separated and dried at room temperature under a stream of nitrogen. The residue was re-dissolved in 100 μL of the mobile phase and 20 μL was injected in the chromatographic system. The running conditions were carried out by using a Waters (Milford, MA, USA) HPLC system, a Luna 5 μ C18 150 mm × 4.6 mm analytical column (Phenomenex, Torrance, CA, USA). The mobile phase was one liter of 2% acetic acid in water:methanol (50:50, v/v) and one bottle of PIC B-7 low UV reagent (size 4 mL) as ion pairing compound, with the pH adjusted to 7.31 with concentrated ammonium hydroxide solution and the UV wave length was 225 nm. The flow rate was 1 mL/min. This method had a low limit of detection of 0.001 μg/mL and the standard curve was linear in the range of 0.05–10 μg/mL with a rate of elimination of correlation of 0.999.

*Pharmacokinetic analysis*

For the compartmental analysis, i.v. plasma levamisole concentration–time profiles, according to AIC criterion (Akaike, 1978) were individually fitted to the following tri-exponential equation:

$$CP = Ae^{-at} + Be^{-bt} + Ce^{-ct}$$

Where *A*, *B* and *C* are the *y* intercept of the extrapolated lines describing tri-exponential were calculated for each subject plasma concentration using the computer program WinNonlin Professional (Pharsight Corporation, Palo Alto, CA, USA).

Due to the complexity of four exponential functions, oral results were not modeled and were evaluated by model independent (noncompartmental) and in order to appropriately compare i.v. and oral results, i.v. pharmacokinetic parameters were also examined by a noncompartmental method. The noncompartmental analyses for the pharmacokinetic parameters after both i.v. and oral administration were determined for each animal using standard formula (Gibaldi & Perrier, 1982). To estimate the extent of absorption each oral subject was matched with an i.v. subject through both periods.

*Statistical analysis*

Data were analyzed by the ANOVA option of Sigma Plot 5 program (SPSS Inc., Chicago, IL, USA). Reported differences in $t_{1/2}$s were based on comparison of the corresponding rate constant. Data were considered significant at 5% ($P < 0.05$).

## RESULTS

Following a single i.v. injection of levamisole (40 mg/kg) at the prelay and at the peak of egg production phases, the concentration time curves followed a three-compartment open model (Fig. 1a,b). The levamisole plasma concentrations after oral administration of levamisole at the prelay and at the peak of egg production are shown in Fig. 2. Levamisole was detected 30 h after both oral and i.v. administration in both of the reproductive periods. The values of pharmacokinetic parameters determined

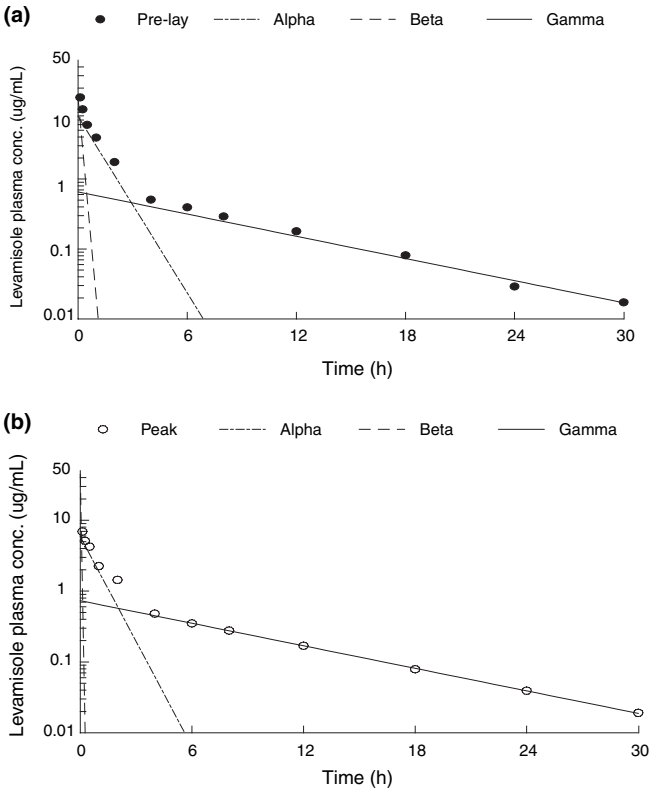


**Fig. 1.** Semilog graphs depicting the mean plasma concentration of levamisole (μg/mL) after i.v. administration of 40 mg/kg at (a) prelay; and (b) peak of egg production.

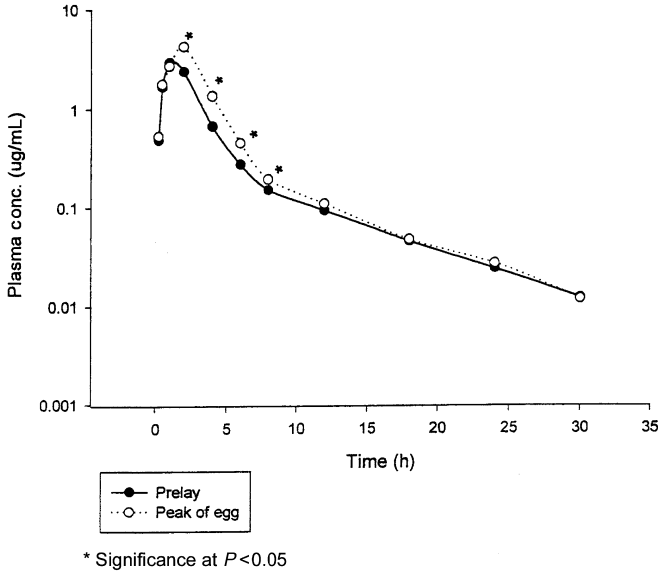


**Fig. 2.** Semilog graphs depicting the mean plasma concentration of levamisole (μg/mL) after oral administration of 40 mg/kg at prelay and peak of egg production. Stars indicate significant differences ($P < 0.05$) between prelay and peak of egg production.

**Table 1.** Pharmacokinetic parameters (mean ± SD) obtained by compartmental analysis after i.v. injection of levamisole (40 mg/kg) in broiler breeder chickens ($n = 10$)

| Parameters | Unit | Prelay phase | Peak of egg production phase |
|---|---|---|---|
| $A$ | μg/mL | 15.4 ± 1.36 | 44.3 ± 22.8 |
| $B$ | μg/mL | 8.50 ± 0.36 | 5.70 ± 0.556* |
| $C$ | μg/mL | 0.700 ± 0.060 | 0.716 ± 0.069 |
| $\alpha$ | /min | 0.122 ± 0.010 | 0.497 ± 0.151* |
| $\beta$ | /min | 0.017 ± 0.001 | 0.018 ± 0.002 |
| $\gamma$ | /min | 0.002 ± 0.0001 | 0.002 ± 0.0001 |
| $AUC$ | μg·min/mL | 985 ± 47.5 | 745 ± 30.9* |
| $MRT$ | h | 3.42 ± 0.095 | 4.26 ± 0.230* |
| $t_{1/2\alpha}$ | min | 5.70 ± 0.518 | 2.09 ± 0.256 |
| $T_{1/2\beta}$ | min | 41.8 ± 2.77 | 39.2 ± 7.82 |
| $t_{1/2\gamma}$ | h | 5.70 ± 0.052 | 5.72 ± 0.230 |
| $Cl_{total}$ | L/kg/min | 0.041 ± 0.002 | 0.053 ± 0.002* |
| $V_{ss}$ | L/kg | 8.36 ± 0.389 | 13.6 ± 1.22* |
| $V_{darea}$ | L/kg | 2.45 ± 0.123 | 3.01 ± 0.675 |
| $K_{21}$ | /min | 0.056 ± 0.004 | 0.078 ± 0.016 |
| $K_{31}$ | /min | 0.003 ± 0.0002 | 0.004 ± 0.0003* |
| $K_{12}$ | /min | 0.048 ± 0.007 | 0.323 ± 0.107* |
| $K_{13}$ | /min | 0.009 ± 0.001 | 0.042 ± 0.016* |
| $K_{10}$ | /min | 0.025 ± 0.001 | 0.067 ± 0.027* |

For explanation of the abbreviations, see Materials and methods.
*Significant difference ($P < 0.05$) between prelay and peak of egg production phases.

by compartmental analysis following i.v. injection of levamisole to chickens in both of the reproductive phases are shown in Table 1 and those calculated by noncompartmental analysis following i.v. administration are shown in Table 2. The values of the noncompartmental pharmacokinetic parameters following oral administration of levamisole 40 mg/kg to chickens in both

**Table 2.** Pharmacokinetic parameters (mean ± SD) obtained by non-compartmental analysis after i.v. injection of levamisole (40 mg/kg) in broiler breeder chickens ($n = 10$)

| Parameters | Unit | Prelay phase | Peak of egg production phase |
|---|---|---|---|
| $AUC_{0-inf}$ | μg·min/mL | 960 ± 45.2 | 694 ± 20.1* |
| $AUMC_{0-inf}$ | μg·min$^2$/mL | 198 344 ± 12 312 | 190 207 ± 6840 |
| $MRT$ | h | 3.44 ± 0.078 | 4.56 ± 0.11* |
| $\beta$ | /min | 0.002 ± 0.0001 | 0.002 ± 0.0001* |
| $t_{1/2el}$ | h | 5.19 ± 0.21 | 5.88 ± 0.40* |

For explanation of the abbreviations, see Materials and methods.
*Significant difference ($P < 0.05$) between prelay and peak of egg production phases.

**Table 3.** Pharmacokinetic parameters (mean ± SD) obtained by non-compartmental analysis after oral administration of levamisole 40 mg/kg in broiler breeder chickens ($n = 10$)

| Parameters | Unit | Prelay phase | Peak of egg production phase |
|---|---|---|---|
| $AUC_{0-inf}$ | μg·min/mL | 599 ± 6.17 | 659 ± 7.89* |
| $AUMC_{0-inf}$ | μg·min$^2$/mL | 145 645 ± 6,444 | 151 217 ± 2,341* |
| $MRT$ | hr | 3.9 ± 0.14 | 3.8 ± 0.06 |
| $\beta$ | /min | 0.002 ± 0.0001 | 0.002 ± 0.0001 |
| $t_{1/2el}$ | hr | 6.06 ± 0.34 | 5.86 ± 0.36 |
| $F$ | % | 61 ± 0.13 | 88 ± 0.26* |

For explanation of the abbreviations, see Materials and methods.
*Significant difference ($P < 0.05$) between prelay and peak of egg production phases.

reproductive phases are given in Table 3. In the i.v. results there was agreement between parameters by noncompartmental and compartmental analysis. This supports the appropriateness of the three-compartmental model.

## DISCUSSION

In this work and based on AIC criterion (Akaike, 1978) the pharmacokinetic profile of levamisole after i.v. administration followed a three-compartmental open body model in chickens. However, levamisole was observed to follow a two-compartment model after i.v. administration in humans (Koussi *et al.*, 1986), pigs and goats (Nielsen & Rasmussen, 1983), sheep (Galtier *et al.*, 1981; Fernandez *et al.*, 1997; Fernandez *et al.*, 1998), goats (Galtier *et al.*, 1981; Sahagun *et al.*, 2000, 2001), rabbits (Garcia *et al.*, 1992) and dogs (Watson *et al.*, 1988) and a one-compartment model after oral and i.m. administration in pigs (Galtier *et al.*, 1983). This difference in the disposition profile may be attributable to the physiological and anatomical differences in those species, and/or differences in the blood sample schedules used in these respective studies.

From the pharmacokinetic analysis of the data collected after i.v. administration, it is concluded that the volume of distribution at steady state ($V_{ss}$), the intercompartmental transfer constants

($K_{12}$, $K_{13}$ and $K_{31}$), the total systemic clearance ($CL_{tot}$) and the elimination rate constant from the central compartment ($K_{10}$) are significantly higher in the phase of peak of egg production than that in the prelay period. This indicates that the reproductive state significantly affects the pharmacokinetic behavior of levamisole in chickens.

The $V_{ss}$ was observably higher in chickens, 8.358 and 13.581 L/kg at the prelay and at the peak of egg production, respectively, than that in other animal species. It was 1.42 L/kg in dog (Watson *et al.*, 1988), 3.88 L/kg in rabbits after i.v. administration (Garcia *et al.*, 1992), 2.14 L/kg in sheep, and 2.76 L/kg in goats after SQ administration (Sahagun *et al.*, 2000). The significant increase in the $V_{ss}$ in the period of peak egg production may be attributed to the high blood lipid level associated with this phase (Christie & Moore, 1972) and with levamisole being a basic organic drug ($p$Ka = 8.0) that has weak lipophilic tendency (Nielsen & Rasmussen, 1983) in an alkaline medium (pH of chicken blood = 7.4). Another case where increased blood lipid enhances intestinal absorption is in HIV-infected patients receiving the lipophilic drug ritonavir (Gursoy & Benita, 2004) and there was an increase in plasma concentrations correlated with cholesterol elevations (De Requena *et al.*, 2003).

The $Cl_{tot}$ is significantly different in the two reproductive stages, 0.041 ± 0.002 and 0.053 ± 0.002 L/kg/min, at the prelay and the peak of egg production periods, respectively ($P < 0.05$). These values are within the range of other animal species, 0.041–0.054 L/kg/min in rabbit after i.v. administration (Garcia *et al.*, 1992), and 0.066 L/kg/min in goats after SQ administration (Sahagun *et al.*, 2000). The half time of elimination ($t_{1/2el}$) is not significantly different in the two reproductive stages (5.69 ± 0.05 and 5.72 ± 0.23 h, respectively) and is close, after i.v. administration, to the 4–5.6 h $t_{1/2el}$ observed in humans (Koussi *et al.*, 1986; Reid *et al.*, 1998). However, it is different, after i.v. administration, than the 0.97 h $t_{1/2el}$ value observed in rabbits (Garcia *et al.*, 1992), the 1.5 h $t_{1/2el}$ observed in sheep and goats after i.v. administration (Fernandez *et al.*, 1997; Sahagun *et al.*, 2001) and 1.8 h $t_{1/2el}$ observed in dogs (Watson *et al.*, 1988) and, after oral administration, the 9.5 h $t_{1/2el}$ value observed in pigs (Galtier *et al.*, 1983).

The results in Fig. 2 and Table 3 indicated more extensive absorption after oral administration during the time of peak of egg production than during the prelay stage. Wang *et al.* (1974) found that the anthelmintic efficacy of levamisole (36 mg/kg body weight, orally) in broilers was 100% for roundworm (*A. galli*) and cecal worm (*H. gallinarum*) and 91% for the intestinal threadworm (*C. obsignata*), while in laying hens, the anthelmintic efficacy was 97% for the roundworm and 99% for the cecal worm. If there were higher blood levels of levamisole in the laying hens than in the broilers in the Wang *et al.* (1974) study, an increase in efficacy would not have been observed because efficacy had already reached maximum (100%) in the broilers. From the noncompartmental analysis of the data collected after oral levamisole dose (Table 3), the area under the curve ($AUC_{0-inf}$) and the bioavailability ($F$%) are significantly higher in the period of peak egg production than that in the prelay period. This may be attributed to an increase in the extent of absorption in the peak of egg production phase. Again this may be because of the lipophilic levamisole being absorbed more readily in the laying birds because of their high blood lipid level that help in carrying the drug from the site of absorption to the blood stream.

Pankavich *et al.* (1973) and Cruthers *et al.* (1975) inoculated pullets with *Capillaria* species and 28 days later found that doses of levamisole 36 mg/kg removed 95–99% of *Capillaria* worms and dose of 48 mg/kg removed 88–90% of *Capillaria* worms (respectively). The observed lowered effectiveness of an increased dose of levamisole in the Cruthers study could be due to variables between the two studies. The Pankavich study used Vantress/Arbor Acre chicks, while the Cruthers study used Babcock chicks. The results of this study demonstrate increased plasma levels in chickens at peak egg production. This could lead to greater efficiency of levamisole in laying hens. It is unlikely that this increased absorption will lead to toxicity because the minimum toxic dose in chickens is 640 mg/kg (Charles & Roberson, 2001).

## CONCLUSION

The levamisole plasma concentration curve followed a three-compartment open model. Egg laying significantly increases the volume of distribution, total body clearance, and bioavailability of levamisole. The increased plasma levels of levamisole may increase efficiency of levamisole in laying hens but would not cause risk of toxicity due to the low toxicity of levamisole in chickens.

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