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SHORT COMMUNICATION

Establishment of Withdrawal Period after Oral Administration of Lincomycin and Colistin Combination in Broiler Chicken

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A B S T R A C T

The combination of lincomycin and colistin is widely used in poultry either for the prevention and treatment of bacterial infection or as additive. However, the presence of antimicrobial residues in food of animal origin is considered as threat to human health. Therefore, this study determined the withdrawal time (WT) by analyzing the residual concentration in tissues to overcome this problem. A combination product of lincomycin and colistin was administered to chickens. Antibiotic residues in edible tissues at different time-intervals were quantified using ELISA and chromatographic methods, and WT was determined. Antibiotics residues were high in the liver and kidney but were lower than the MRL on day 1 and rapidly decreased from day 3. The WT in the kidney was calculated as 2.95 days and is rounded off to 3 days. This study suggested the withdrawal time of 3 days as a precautionary principle for public health.

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INTRODUCTION

The commercial poultry industry is developed well globally, and this industry supplies the largest portion of animal protein in the form of eggs and meat. Antimicrobials are used by poultry veterinarians and poultry industries for reducing bacterial infections, and enhancing feed efficiency and growth (Donoghue, 2003). Antimicrobial usage has facilitated the efficient poultry production along with the reduction of bacterial disease outbreaks, allowing the consumer to buy high quality eggs and meat at a reasonable price. But, antimicrobial residues in edible tissues and eggs may directly cause toxic reactions in consumers or may indirectly initiate problems by inducing resistant development in bacteria (Stolker and Brinkman, 2005). Therefore, the presence of antimicrobial residues in food of animal origin is currently considered as human health hazard and the maximum residue limits (MRL) is addressed for considering the safety of consumers.

Colistin is used as an animal additive in Asia and mainly for preventing and treating intestinal bacterial-infections in pigs, poultry, rabbits, cattle, sheep and goats (Kempf *et al*., 2016; Jamal *et al*., 2017). Combinations of colistin with other antimicrobials are available for treatments of bacterial infections in food-producing animals in many countries including some European Member States. The combination of colistin and lincomycin is one of these combination antimicrobials used widely. Lincomycin is effective against *Staphylococcus aureus*, *Streptococcus viridans*, *Leptospira pomona*, *Diplococcus pneumoniae*, β-hemolytic *Streptococcus* and Mycoplasma infection. Despite the increased-use of lincomycin and colistin combination, there is no information on the exact withdrawal time (WT). Therefore, in this study, we intend to establish the WT by analyzing the tissue residual concentration after administering a combination product of colistin and lincomycin to chickens.

MATERIALS AND METHODS

Preparation of antibacterial combination: Standard solutions of antimicrobials were prepared by dissolving 1 mg of each of lincomycin and colistin (Sigma-Aldrich, St. Louis, MO, United States) in 100 mL of acetonitrile to make a concentration of 10 μg/mL. The combination antimicrobial solution used in this study (Lincol, SHINIL Biogen Co., Ltd, Anyang-si, Republic of Korea) is composed of 100 g of lincomycin and 600,000,000 IU of colistin.

Animal experimental procedure: The F1 cross-bred of Landrace female and Large White male with the body weight of 360-500 g (2-3 weeks old) was employed in this study. Lincol at the recommended dose (1 mL) and thrice of the recommended dose (1 mL × 3) were orally administered for 5 days, while the day of drug-administration was considered as day 0. Liver, kidney, muscle, skin and fat were collected after euthanizing chickens at 0, 1st, 3rd, 5th, 7th and 14th days of post-drug administration, and residual concentrations of lincomycin and colistin were determined. To estimate the pharmacokinetic breaks, Lincol was administered to chickens at recommended dose of lincomycin for 3 days. Blood samples were collected at different times after final dose administration. Approval for the protocols of animal experiments was obtained from Kyungpook National University's Animal Care and Use Committee (approval number KNU 2016-0013).

Analysis of drug residues in blood and tissues: Colistin and lincomycin were quantified by MaxSignal ELISA kits (Bioo Scientific Corp. Austin, TX, United States) according to instructions of manufacturer. Again, lincomycin and colistin were analyzed by HPLC, and quantification of lincomycin was reconfirmed by LC/MS/MS. Edible tissues of 1–2 g of each were separately analyzed to determine lincomycin and colistin residues.

Determination of withdrawal time: The WT of the test substance was determined by considering the recommended MRL mentioned in *European Agency for the Evaluation of Medicinal Products* (EMEA) and "Animal Product Residue Chemical Risk Assessment Manual" in Republic of Korea (RAMK). The duration of pharmacokinetic breaks and linear regression analysis (LRA) were used to determine WT. LRA of logarithmic transformed tissue concentrations set the WT to the point at which the residual concentration of tissue fell to a concentration below the MRL within the 95% confidence interval.

RESULTS AND DISCUSSION

It is important and necessary to determine the drug residues in edible tissues, since edible tissues are the major source of protein uptake from chicken. Based on the guidelines of FDA, the target tissues for evaluating the WT should be those edible tissues from which residues eliminate most slowly. Alongside, EMEA (2002) mentioned liver, kidney and muscle as target tissues for poultry. Moreover, the selection of appropriate analysis techniques and their validation should be accomplished before studying the drug-tissue depletion for determining WT. In this study, we selected ELISA and chromatographic assay methods to quantify residues. Critical factors of this study were high specificities and accuracies of analytical methods. Analytical methods used in this study were valid (Data not shown).

According to the recommendation of EMEA and RAMK, the MRL of colistin were 150, 150, 150 and 200 ppb, and the MRL of lincomycin were 100, 50, 500 and 1500 ppb in muscle, skin+fat, liver, and kidney of chicken, respectively. Residues of lincomycin in different tissues were found after 1, 3 and 5 day of administration by ELISA assay (Fig. 1). The residue of lincomycin only in skin+fat after 1 day of administering Lincol (1 mL) was more than 2-times of the MRL. The lincomycin residues in liver, muscle and skin+fat tissues after 1 day of administering Lincol (3 mL) were higher than the MRL. By LC-MS/MS analysis, the lincomycin residues in kidney and skin+fat tissues after 1 day of administering the recommended dose were higher than the MRL (Table 1). It was found that the residue of colistin in skin+fat tissue was more than the MRL after 1 day of administering the recommended dose by ELISA assay (Fig. 1). However, residues of colistin in all tissues after 1 day of administering Lincol (3 mL) were higher than the MRL. Both antibiotics were found to have residual tissue levels below MRL on day 3 after administering the recommended dose. The results of ELISA analysis showed that residues of both drugs were high in the liver and kidney of chicken treated with recommended dose, but were lower than the MRL on day 1, rapidly decreased from day 3 and were found to be negligible on day 5.

Table 1: LC-MS/MS analysis to confirm presence of lincomycin in different tissues after administering the recommended dose

Tissues	After treatment (day)			
(MRL, ppb)	1	3	5	7
Kidney (1500)	1871±113	960±102	-	-
Liver (500)	251±31	175±18	-	-
Muscle (100)	55±12	26±2	-	-
Skin+Fat (50)	114±34	48±5	-	-

The results of the duration of pharmacokinetic breaks are shown in Fig. 2A. Lincomycin concentration in the plasma reached the maximum (7.58 μg/mL) after 30 minutes of oral administration and then gradually decreased. The biological half-lives of healthy broiler chickens were 1.27 h. Based on the FARAD standard, the WT is calculated to be ten times the half-life period. Therefore, most lincomycin was eliminated from the body by 12.7 h. However, since lincomycin is absorbed and distributed in the body, setting of one day as WT may be a residual risk. It is found that tissues take more time than blood to absorb the drug. As tissues take more time for absorbing lincomycin, they also take longer for eliminating or metabolizing the drug. These data also justify the determination of lincomycin WT in chicken's edible tissues. WT in this study was only based on the residual concentration of lincomycin as lincomycin had comparatively higher tissue residual concentration for longer period of time than colistin.

The WT was calculated by LRA of the time-concentration change of lincomycin in the kidney by thrice of the recommended-dose. The WT in the kidney was calculated as 2.95 days and is rounded off to 3 days (Fig. 2B). The regression method is considered to be an outstanding approach for estimating WT. To calculate WT, the EMEA (1995) recommends for performing a LRA of the logarithmic-transformed concentrations of drugs and/or their metabolites during the tissue-depletion stage. The WT of lincomycin and colistin were calculated at the point where the upper 99% tolerance limit for the residue is below MRLs with 95% confidence. According to the Codex Alimentarius Commission (1995), the lengths

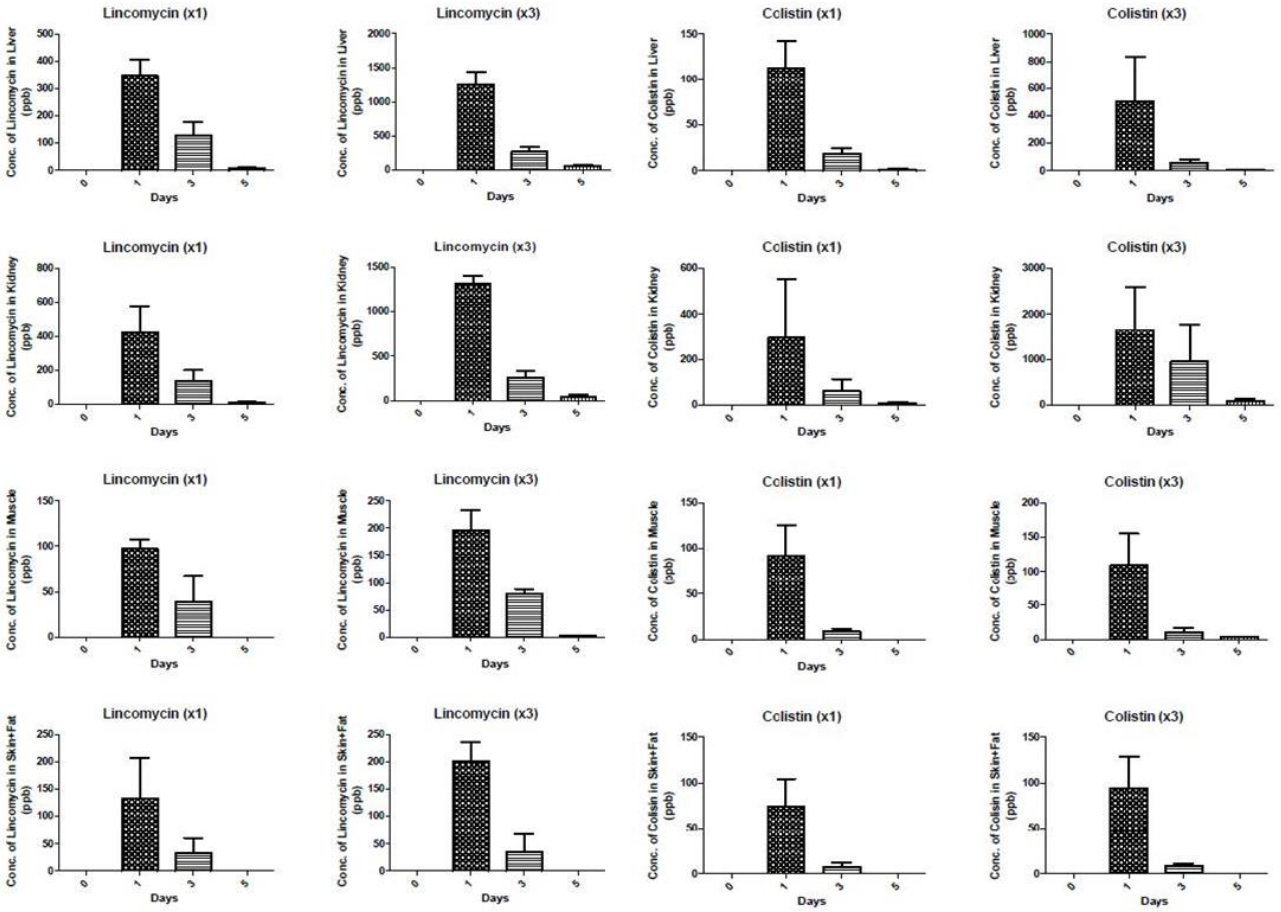

Fig. 1: Identification of lincomycin and colistin residues in tissues by ELISA.

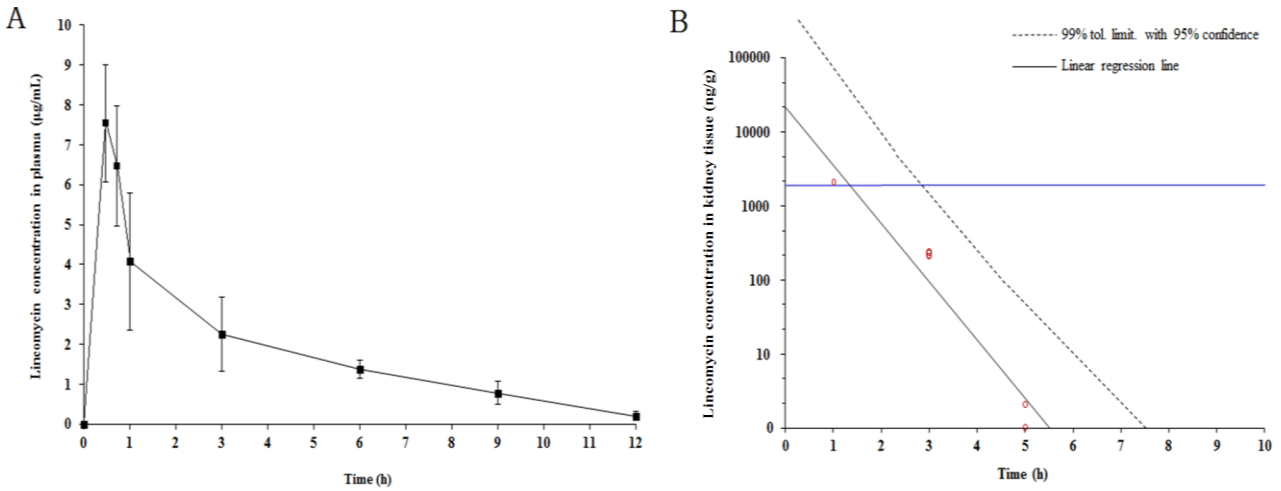

Fig. 2: Withdrawal time estimation based on (A) pharmacokinetic analysis and (B) linear regression analysis of residual lincomycin respectively in plasma and kidney tissue.

of WT are determined by MRL. In this investigation, WT of lincomycin and colistin were calculated based on the MRLs mentioned by EMEA and RAMK. It is important to note that concentrations of both drugs in liver, kidney, skin+fat and muscle were lower than the MRLs in most of the cases.

Conclusions: It is possible to suggest that a withdrawal time of 3 days could be assigned as a precautionary principle for public health, without a significant economic impact for broiler producers.

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Authors contribution: SCP conceived and designed the study and revised the manuscript. NHP, RP, and MAH were participated in experiments, data analysis and

manuscript draft preparation. All authors critically revised the manuscript and approved the final version.

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