J Vet Clin 27(5) : 565-568 (2010)

Journal of Veterinary Clinics
www.ksvc.or.kr

Residue Depletion of the Sulfaquinoxaline and Trimethoprim Combination in Broilers

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(Accepted: January 05, 2010)

Abstract : Sulfaquinoxaline (SQX) and trimethoprim (TMP) are chemotherapeutics that are extensively used in various animal species for the treatment or prevention of coccidia and coccidia-like parasites. Little information about the depletion kinetics of these compounds in chickens exists in the literature. In this study, a new commercial liquid concentrate of SQX in combination with TMP (100 g/l of SQX and 33.4 g/l of TMP) was administered with drinking water at a dose of 0.75 ml/l or 1.5 ml/l. The edible tissue concentrations of the drugs were determined by the validated high-performance liquid chromatography/mass spectrometric method. Residue concentrations of SQX and TMP were lower than their maximum residual limits (MRLs) in all tissues from both dose groups at 5 days after the treatment. The optimal withdrawal time of SQX/TMP combination was suggested to be over 5 days after cessation of medication in broilers.

Key words **:** sulfaquinoxalin, trimethoprim, broiler, residues.

Introduction

Sulfonamides used in veterinary medicine for the treatment or prevention of coccidia and coccidia-like parasites include sulfagunidine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamethazine, sulfamethoxazole, sulfanitran and sulfaquinoxaline (5). Sulfonamides have more activity against the asexual stages and lesser activity against the sexual stage of coccidia. Because of drug resistance and development of new broad-spectrum anticoccidials, no sulfonamides were marketed as single feed additives for the prevention or treatment of coccidiosis (5,7). Many are used in combination with other chemotherapeutics and they are also still widely used in the treatment of coccidiosis in animals (5).

Sulfaquinoxaline/trimethoprim regimens (3:1) in diet or in the drinking water were widely used in treatment of pasteurellosis, colisepticaemia and coccidiosis at a total dose of 30 mg/kg/day (9,10,12). The aim of this study was to evaluate the residue depletion profiles of a new commercial formulation, sulfaquinoxaine (SQX)/trimethoprim (TMP) combination liquid concentrate, after its treatment via drinking water for 3 consecutive days. Information about the relative disposition of these compounds in the target species is important to determine their treatment schedules and estimation of withdrawal periods that can provide the best chemotherapeutic efficacy and regulatory frames.

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Materials and Methods

Animals

The experiment was conducted in farm housing broilers of around 1 kg body weight. SQX/TMP combination liquid concentrate (100 g/l of SQX and 33.4 g/l of TMP; Coccimyul®, DaeHanNewPharm, Seoul, Korea) was given for 3 consecutive days in drinking water at a dose of 0.75 ml/l (recommended dose) or 1.5 ml/l (high dose) to broilers. Six broilers were taken at random and killed before the start of the experiment and 0, 2, 5 and 7 days after the last dose. Samples of liver, kidney, muscle, skin + fat and serum were collected and stored in the freezer at −50°C and allowed to thaw at room temperature before processing.

Sample preparation

The extraction of SQX and TMP in poultry tissues was carried out by the liquid-liquid extraction with methanol and n-hexane. In short, each 1 g sample was added to 5 ml of warm methanol and homogenized, and then shaken for 5 min. The homogenized samples was centrifuged at 1,300 *g* for 10 min, the upper phase being transferred into other tubes and evapo-

rated to dryness under a stream of nitrogen. The residue was reconstituted with 1 ml of acidic buffer solution (pH 4.5, sodium acetate and acetic acid) and then 0.5 ml of n-hexane was added for extraction of SQX and TMP. After vortexing for 5 min, the sample was centrifuged at 500 *g* for 10 min and the lower phase being transferred into other tubes. To this sample was added 1 ml of chloroform for removal of lipid. The vortexed sample for 5 min was centrifuged at 500 *g* for 10 min, the lower phase being transferred into other tubes and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 1 ml of methanol and then aliquot of 10 μl after filtration was injected on column.

Instrumental analysis

Samples were analyzed on a Agilent 1100 series LC/MSD system. Separation was achieved on Nova-Pak C_{18} reverse phase column (4 μm, 3.9 mm × 150 mm, Waters, USA). Flow rate was operated isocratically at 0.6 ml/min. The mobile phase consisted of 50 mM ammonium acetate and acetonitrile (2:8, v/v). The mass spectrometer was run in the positive mode and

Table 1. Residual concentration of SQX and TMP in various tissues obtained at different time points after treatment of SQX/TMP combination liquid concentrate (100 g/l of SQX and 33.4 g/l of TMP) via drinking water at dose of 0.75 ml/l for 3 consecutive days in broilers

Durgs	Tissues	Tissue concentrations ($n = 6$; μg/g) after treatment for 3 consecutive days			
		Day 0	Day 2	Day 5	Day 7
Sulfaquinoxaline	Skin + fat	0.73 ± 0.01	0.24 ± 0.01	-	-
	Serum	3.01 ± 0.07	0.47 ± 0.02	0.03 ± 0.01	-
	Kidney	2.47 ± 0.05	0.31 ± 0.01	0.01 ± 0.01	-
	Liver	2.17 ± 0.02	0.25 ± 0.03	-	-
	Muscle	1.07 ± 0.02	0.22 ± 0.01	-	-
Trimethoprim	Skin + fat	0.11 ± 0.00	0.04 ± 0.01	-	-
	Serum	0.45 ± 0.04	0.08 ± 0.00	-	-
	Kidney	0.35 ± 0.01	0.09 ± 0.00	-	-
	Liver	0.33 ± 0.01	0.06 ± 0.01	-	-
	Muscle	0.12 ± 0.01	0.03 ± 0.00	-	-

†Data were expressed by mean ± standard deviation (SD).
-, not detected or under limit of quantitation (LOQ).

Table 2. Residual concentration of SQX and TMP in various tissues obtained at different time points after treatment of SQX/TMP combination liquid concentrate (100 g/l of SQX and 33.4 g/l of TMP) via drinking water at dose of 1.5 ml/l for 3 consecutive days in broilers

Drugs	Organs	Tissue concentrations ($n = 6$; μg/g) after treatment for 3 consecutive days			
		Day 0	Day 2	Day 5	Day 7
Sulfaquinoxaline	Skin + fat	1.40 ± 0.03	0.61 ± 0.02	0.01 ± 0.01	-
	Serum	5.97 ± 0.07	1.04 ± 0.02	0.06 ± 0.02	-
	Kidney	4.20 ± 0.11	0.57 ± 0.04	0.03 ± 0.01	-
	Liver	3.26 ± 0.27	0.49 ± 0.01	0.01 ± 0.01	-
	Muscle	1.70 ± 0.03	0.36 ± 0.02	-	-
Trimethoprim	Skin + fat	0.23 ± 0.01	0.08 ± 0.01	-	-
	Serum	0.84 ± 0.02	0.14 ± 0.01	0.01 ± 0.01	-
	Kidney	0.71 ± 0.03	0.14 ± 0.01	-	-
	Liver	0.69 ± 0.03	0.13 ± 0.01	-	-
	Muscle	0.22 ± 0.00	0.06 ± 0.01	-	-

†Data were expressed by mean ± standard deviation (SD).
-, not detected or under LOQ.

selective ion monitoring mode focused on $m/z = 291.5$ for TMP and $m/z = 301.5$ for SQX.

Analytical validations

The calibration curve was linear in range of 0.005-10 μg/ml for SQX and TMP with a correlation coefficient of 0.99 and 0.99, respectively. Precision and accuracy met certain criteria for the guideline of residual analysis of veterinary drugs in National Veterinary Research and Quarantine Service (NVRQS, 8). The applicability of the new method was successfully demonstrated in edible tissues of broilers from two residue depletion studies.

Results and Discussion

Residue concentrations were associated with administered dose (Table 1 and 2). At the termination of treatment, SQX and TMP were found in all collected samples for both dose groups. The ratio of SQX-TMP in serum and tissues were shown over the optimal ratio of SQX-TMP of 3:1 in the present study. In the 5 day continuous treatment of sulfadiazine (SDA)-TMP combination, an average ratio of 80:1 was found throughout the treatment in spite of their optimal ratio 20:1 (2). In addition, Löscher *et al.* (6) and Dagorn *et al.* (1) reported that tissue ratios of SDA:TMP also differed from this optimal ratio at 1 day after cessation of medication, being 4.7:1 in the skin + fat, 60-85:1 in the plasma and 5:1 in the lung after continuous administration of SDA and TMP in broilers.

Li *et al.* (4) reported that the half-life of SQX was the shortest in the muscle, with longer half-lives in the heart, plasma, liver and kidney after a single 200 mg/kg oral dose of SQX in broilers. These results are consistent with our results. The highest residual concentration of SQX was shown in serum, followed by kidney, liver, muscle and skin after oral dose of SQX-TMP combination for 3 consecutive days in broilers (Table 1 and 2). TMP is a lipid-soluble organic base that distributes to most tissues of the body and tends to concentrate in tissues with a greater acidity than plasma (11). However, the highest concentration of TMP was found in serum, followed by kidney, liver, muscle and adipose tissue similar to SQX (Table 1 and 2). It was suggested that higher serum residual concentrations than other editable tissues were related from their higher recovery in comparison with recoveries of other edible tissues.

According to the veterinary drug residue regulations of the NVRQS, the maximum residue levels (MRLs) of SQX and TMP in broiler tissues are 0.1 mg/kg, 0.05 mg/kg, respectively (3). Residue concentrations of SQX and TMP were lower than each LOQ in all tissues from both dose groups (recommended dose and high dose) at 7 days after the treatment.

In conclusion, the optimal withdrawal time of SQX/TMP combination was suggested to be over 5 days after cessation of medication at a dose of 0.75 ml/l or 1.5 ml/l of drinking water in broilers.

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육계에서 설파퀴녹살린 및 트리메토프림 합제의 잔류분석

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요 약 : 설파퀴녹살린과 트리메토프림 합제는 닭콕시듐병의 예방과 치료목적으로 널리 사용하고 있으나, 닭에서의 잔류에 관한 연구보고는 미흡하다. 본 연구에서는 음수첨가용 액제로 신규 개발된 설파퀴녹살린 및 트리메토프림 합제 (설파퀴녹살린, 100 g/L; 트리메토프림, 33.4 g/L)를 육계에 음수 리터당 본제 0.75 mL 및 1.5 mL를 3일간 투여한 후 살처분하여 가식부위내 잔류농도를 액체크로마토그래프/질량분석기를 이용하여 측정하였다. 그 결과 약물 투여 후 7일째에는 모든 가식부위내에서 설파퀴녹살린 및 트리메토프림은 검출되지 않았다. 따라서 신규 음수첨가용 액제인 설파퀴녹살린 및 트리메토프림 합제의 휴약기간은 최소 5일 이상으로 설정되어야 할 것이다.

주요어: 설파퀴녹살린, 트리메토프림, 육계, 잔류