

Sulfadimethoxine Residue in Broiler-Chicken Skin

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ABSTRACT. The disposition and elimination of sulfadimethoxine (SDMX) in the skin of broiler-chickens were investigated. The administration of SDMX, in drinking water, at a concentration of 1,000 ppm for 5 days demonstrated that the SDMX was eliminated much more slowly from the skin than from the other tissues or plasma. These results were duplicated and confirmed in another experiment, in which a single dose of 200 mg/kg BW of SDMX was administered via a stomach tube. No significant difference in the SDMX residue level was observed between the broiler-chickens that had their skin surface sealed versus the non-sealed animals and that had ingested SDMX in their drinking water. This illustrated the higher SDMX residue in the skin was not attributable to external SDMX contamination from the drinking water, feces or urine. In addition, there was no significant difference among the SDMX residue concentrations in the thoracic, dorsal and leg skin samples, following an intravenous injection of SDMX (30 mg/kg BW). This indicated that the SDMX was distributed evenly throughout the entire skin area of the broiler-chickens.—**KEY WORDS:** chicken, drug residue, skin, sulfadimethoxine.

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Sulfa drug residues in domestic animals have been reported to remain longer in the kidney [1, 3, 6] and the plasma [4, 5] than in other tissues. This longer period of drug residue detected in the kidney may be caused by concentrating these drugs into the organ prior to excretion [7], while the prolonged residue retention in the plasma might be attributable to the strong protein binding characteristics of these drugs [2].

In this study, the sulfadimethoxine (SDMX) residues in various tissues, especially in the skin of broiler-chicken were investigated. This information is important for public health to protect consumers from the drug residue contamination since SDMX is often used as an anticoccidial drug for broiler-chicken in Japan, and the skin of the animal is considered as an edible tissue. The source of SDMX residue in broiler-chicken skin was also investigated.

MATERIALS AND METHODS

Animals: Forty two female broiler-chickens (Arbor Acre), forthwith referred to as broilers, weighing from 1.1 to 1.9 kg were used in this experiment. Three to five animals were housed in each wire floor cage. Commercial feed and tap water were provided *ad libitum*.

Drug administration: Drugs were administered by the following three methods. (1) SDMX powder was dissolved in tap water at a concentration of 1,000

ppm. The SDMX solution was provided on an *ad lib.* basis to 25 broilers for 5 days, replacing their drinking water. Ten of the broilers were treated in the following manner during this experiment. The right thoracic region was covered with a sheer bandage (waterproof type, 50 × 100 mm. Aso Pharmaceutical Co., Ltd., Japan) and the margin of the bandage was sealed to the skin using gum tape and adhesives. (2) SDMX Injection was diluted to half concentration with deionized water and administered to 12 broilers at a single oral dose of 200 mg SDMX/kg body weight via a stomach tube. This dose was decided to be almost similar to the mean daily intake amount of SDMX calculated in the experiment (1). (3) The SDMX Injection was given intravenously at a dose of 30 mg SDMX/kg body weight to 5 broilers.

Sampling: The broilers that received SDMX in their drinking water were sacrificed 1, 3, 5, 7 and 10 days after the withdrawal of the SDMX. The sacrificed broilers were dipped in 60 to 70°C water for about 10 sec and the thoracic feathers were completely removed by hand. Samples from the thoracic skin, kidney, liver, muscle, fat, small intestine and plasma were obtained. The skin of the thoracic region was obtained almost free from subcutaneous fat. The skin samples were flushed with tap water and placed onto a filter paper to blot the water. The broilers that received SDMX via the stomach tube were sacrificed 1, 3, 5 and 10 days post

the SDMX administration and samples were collected as described above. The broilers that were injected intravenously with SDMX were sacrificed 6 hr after the administration to obtain the skin samples from the thoracic, dorsal and leg regions. The skin samples from the thoracic and leg regions were obtained almost free from subcutaneous fat. The subcutaneous fat found under the dorsal skin samples was removed using scissors. All samples were stored in a deep freezer at -20°C until they were analyzed.

Drug analysis: (1) Five grams of the tissue samples were placed in a 50 ml centrifuge tube and homogenized with 30 ml of acetonitrile for 1 min (four to five grams of anhydrous sodium sulfate were further added in case of the fat samples). This homogenate was centrifuged at 2,500 r.p.m. for 5 min to separate the supernatant. The residue was shaken with 30 ml of acetonitrile for 10 min and then centrifuged to obtain the supernatant. These two supernatants were combined, then placed in a 200 ml flask with 10 ml of n-propylalcohol and evaporated to complete dryness *in vacuo* at 40°C . Two milliliters of mobile phase and a few glass beads were added to the residue, then the flask was placed in an ultrasonic cleaner box for 1 min with manual rotational movement to detach the residue from the surface of the glass. This sample solution was filtered through a filter disk (pore size of $0.45\ \mu\text{m}$). Thirty microliters of this filtrate were injected into a High Performance Liquid Chromatograph (Japan Spectroscopic Co., Ltd.) under the following conditions. Column: Zorbax ODS (4 mm I.D. \times 150 mm), mobile phase: Acetonitrile/water/acetic acid mixture (33/67/0.1), flow rate: 1.0 ml/min, detection wavelength: 270 nm, range: 0.04. The peak heights of the SDMX on the chromatogram were measured and the SDMX concentration was then calculated employing a standard curve. (2) Three milliliters of the plasma samples were shaken vigorously with 3 ml of ethylalcohol, 20 ml of 0.2 M phosphate buffer (pH 5) and 30 ml of ethylacetate for 10 min. This mixture was then centrifuged to separate the upper layer. Thirty milliliters of ethylacetate were added to the lower layer and this procedure was repeated. The combined upper layers, along with 10 ml of n-propylalcohol were evaporated to dryness. One milliliter of mobile phase and a few glass beads were added to the residue and processed by the same procedure described for tissue samples.

Drugs: SDMX powder (Abcid Soda Powder, 100

mg/g, Daiichi Pharmaceutical Co., Ltd., Japan) and SDMX Injection (Abcid Injection, 200 mg/ml, Daiichi Pharmaceutical Co., Ltd.) were used to administer the SDMX.

Statistics: Statistical analysis was performed using paired Student's *t* test. A P value less than 0.05 was considered to be statistically significant.

RESULTS

The analytical method applied in this study was able to detect the SDMX residue levels of $0.05\ \mu\text{g/g}$ for tissue or $0.05\ \mu\text{g/ml}$ for plasma. The recovery rates for SDMX when added to the control tissues and plasma at a concentration of $0.8\ \mu\text{g/g}$ or ml were 84 to 95% (C.V. 2 to 5%, $n=5$).

The SDMX provided to the broilers in their drinking water for 5 days disappeared rapidly from both the plasma and all of the tissue samples excluding the skin samples (Fig. 1). Meanwhile, the SDMX residue, which disappeared slowly from the skin samples, could be detected up to 10 days after the withdrawal of the SDMX. The daily intake amount of the SDMX was calculated to be 188 ± 30 mg/kg body weight (mean \pm S.D., $n=25$).

The SDMX administered at the single oral dose of

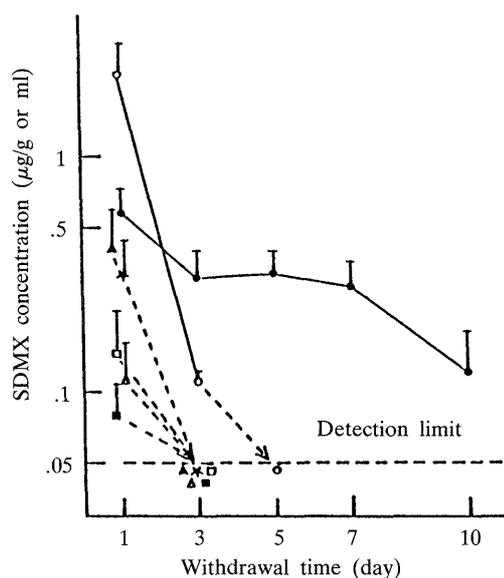


Fig. 1. Residual concentrations of SDMX in broiler tissues after the oral administration through drinking water at a concentration of 1,000 ppm for 5 days. ● skin, ○ kidney, ▲ liver, △ muscle, ■ fat, □ small intestine, and ★ plasma. (mean \pm S.D., $n=3$)

200 mg/kg via the stomach tube also disappeared rapidly from both the plasma and the tissue samples except the skin (Table 1). The SDMX residue could be detected in skin samples up to 5 days post the SDMX administration. The SDMX concentration in the skin samples one day following the SDMX administration was lower than those in the plasma and kidney samples, but comparable to the concentrations found in the liver, muscle and small intestine samples, and higher than those in the fat samples (Table 1).

Table 1. Residue levels of SDMX in broiler tissues after the single oral administration (200 mg/kg) via a stomach tube

Sample	Withdrawal period (day)			
	1	3	5	10
Skin	48±9 ^{a)}	0.12±0.05	0.13±0.09	<0.05
Plasma	131±22	0.09 ^{b)}	<0.05	<0.05
Kidney	103±16	0.25±0.22	<0.05	<0.05
Liver	54±9	0.06 ^{b)}	<0.05	<0.05
Muscle	40±11	<0.05	<0.05	<0.05
Fat	16±2	<0.05	<0.05	<0.05
Small intestine	34±4	<0.05	<0.05	<0.05

a) Mean±S.D. ($\mu\text{g/g}$ or ml), $n=3$.

b) The figure represents the value of one sample. Values of the other two samples were under the detection limit ($0.05 \mu\text{g/g}$ or ml).

Table 2. Comparison of SDMX residue levels between sealed (right thorax) and non-sealed (left thorax) skins after the oral administration through drinking water at a concentration of 1,000 ppm for 5 days

Animal No.	Withdrawal period (day)	SDMX concentration ($\mu\text{g/g}$)	
		sealed skin	non-sealed skin
1		0.66	0.67
2		0.74	0.66
3	1	0.34	0.37
4		0.32	0.40
5		3.16	3.28
6		0.28	0.40
7		0.33	0.29
8	3	0.19	0.23
9		0.21	0.21
10		0.71	0.58

Table 3. Comparison of SDMX concentrations between thoracic, dorsal and leg skins obtained 6 hr after the intravenous injection (30 mg/kg)

SDMX concentration ($\mu\text{g/g}$)		
Thorax	Dorsum	Leg
12.3±0.5 ^{a)}	11.5±1.7	12.0±1.0

a) Mean±S.D. ($n=5$)

There was no significant difference between the SDMX residue levels found in the sealed and non-sealed skin samples (Table 2).

Also, no significant difference in the SDMX concentrations between the thoracic, dorsal and leg skin samples obtained 6 hr after the intravenous injection was observed (Table 3).

DISCUSSION

The results obtained from this study demonstrated that the SDMX residue remained detectable longer in broiler skin than in other tissues and plasma (Fig. 1, Table 1). The drug residue in broiler skin is an important public health concern because broiler skin is considered as an edible tissue and comprises $13.3\pm 0.8\%$ of the carcass weight (body weight: 1.96 ± 0.25 kg, $n=5$, not published). The SDMX residue also persisted in the kidney samples, but much less in the fat, liver, muscle and small intestine samples (Fig. 1). These results differ from the residue study with laying hens [7], which reported that the SDMX residue was detectable for a comparable period in the skin samples with subcutaneous fat, peritoneal fat, kidney and plasma samples. The reason for the difference between these two experiments could not be explained.

As shown in Table 2, the SDMX skin residue was not due to the external contamination of the drug in drinking water, feces or urine, but resulting from the SDMX absorbed into the body. The initial distribution rate of SDMX to the skin was similar to or lower than the distribution rates to the other tissues and the plasma (Table 1). There was no significant difference between the SDMX concentrations in the thoracic, dorsal and leg skin samples (Table 3). These results suggested that the SDMX was distributed evenly throughout the whole skin, with no preferential sites detected.

The details of the mechanism for SDMX residue in broiler skin is still under investigation from the perspective of the pharmacokinetics and comparison with other drugs.

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