Pharmacokinetics of Sulfadimethoxine in Skin of Broiler-Chicken after Single and Multiple Intravenous Injections

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ABSTRACT. Pharmacokinetics of sulfadimethoxine (SDMX) in skin of broiler chicken after intravenous and in-drinking-water administrations were investigated to examine the reason for a longer residue of SDMX in the skin which was observed in the residue study after administration via drinking water at a concentration of 1,000 ppm. The decay curve of SDMX in the skin after single intravenous injection of 200 mg/kg, the highest dose, was fitted to the two compartment model with T1/2 of 4.4 hrs in the first elimination phase and 173 hrs in the second one. The extrapolated concentrations in the skin at 24 hrs after the injection were calculated to be 69.0 µg/g for the 1st phase and 0.11 µg/g for the 2nd phase. The decay curve in the skin after single injection of 30 and 100 mg/kg were fitted to the one compartment model with T1/2 of 3.2 and 5.7 hrs, respectively. Dividing a high dose into 3 to 5 doses and injecting sequentially with intervals of the previously measured T1/2, SDMX concentrations in the skin were about half of those in the plasma and ran parallel. The plasma concentration-time curves after single intravenous injection of SDMX more than 100 mg/kg showed nonlinear kinetics with concentrations over 100 µg/ml for 12–30 hrs after the injection. By administration of SDMX via drinking water, a sustained residue curve of SDMX in the skin at 1,000 ppm reported previously was not observed at 500 ppm. These results suggest that existence of two compartments for SDMX in the skin, i.e. the concentration in the first compartment ran parallel with that in plasma and only a slight portion of plasma SDMX was uptaken by the second compartment to be eliminated extremely slowly.—KEY WORDS: chicken, pharmacokinetics, skin, sulfadimethoxine.

Sulfadimethoxine (SDMX), a long-acting sulfonamide, is used for treatments of bacterial infections and coccidiosis in domestic animals. In the previous paper, it has been shown that SDMX administered through drinking water was eliminated more slowly from skin than from any other edible tissue or plasma of broiler-chickens [7].

In this study, the pharmacokinetics of SDMX in the broiler-chicken skin after single and multiple intravenous administrations of the drug was investigated to confirm the extremely slow elimination from the skin. The residue study of SDMX in broiler-chickens after administration through drinking water was also performed to determine the appropriate drug concentration in water to avoid a long residue in the skins.

MATERIALS AND METHODS

Animals: Eighty four female broiler-chickens (Arbor Acre), weighing from 1.3 to 2.5 kg, were used. Three to six animals were housed in a wire floor cage, allowed to take commercial diet (Super Goal, Taiyo Feed Co., Ltd., Japan) and tap water ad lib.

Drugs: SDMX Injection (Abcid Injection, Daiichi Pharmaceutical Co., Ltd., Japan) and SDMX Powder (Abcid Soda Powder, Daiichi Pharmaceutical Co., Ltd.) were used.

Drug administration: Drug was administered by one of the following three methods. 1) Single intravenous injection of SDMX at a dose of 20, 30, 40, 100 or 200 mg SDMX/kg body weight. 2) The multiple intravenous injections according to the protocol shown in Table 1. The single and multiple injections were started at 10:00 a.m. 3) Administration via drinking water for 5 days. The solution was prepared by dissolving SDMX Powder in tap water at a concentration of 500 ppm, a common therapeutic dose.

Sampling: The broilers received single intravenous injection at a dose of 30, 100 or 200 mg/kg were sacrificed 0.25, 1, 1.5, 2, 3, 5 and 7 days after injection to obtain the thoracic skin and plasma according to the method described in the previous paper [7]. From the broilers injected repeatedly, both the skin and plasma from a scheduled portion of the broilers were obtained just before next administration and 3.5 or 10.3 hrs after the final one (Table 1). After removing the thoracic feathers by
hand, 50–100 mg of a skin sample was obtained by cutting approximately 1 cm² of the skin by scissors. Local anesthesia with 0.2 ml of 2% procaine was performed just before the sampling according to the guideline for animal experiments established in our institute. The wound of the skin was closed with one or two stitches and disinfected with 70% ethylalcohol. The following sampling was performed from a site more than 3 cm apart from the site sampled before. The skin samples were obtained almost free from subcutaneous fat. The sample was rinsed in saline and placed onto a filter paper to blot the saline. After weighing, the sample was stored in 10 ml of acetonitrile in polyethylene tube. Approximately 1 ml of plasma was obtained from 2 ml of blood collected just after the skin sampling. For the plasma kinetic study, 2 ml of blood was collected at the appropriate time intervals shown in Fig. 3 after single injection of 20, 40, 100 and 200 mg/kg to obtain the plasma. The broilers which received SDMX via their drinking water were sacrificed 1, 3, 5 and 7 days after the withdrawal in order to obtain the thoracic skin, kidney, liver, muscle, fat, small intestine and plasma. All samples were stored in a deep freezer at −20°C until analysis.

Drug analysis: The SDMX concentration in the samples was determined by HPLC [7].

Serum protein binding: SDMX was added at a concentration of 1, 10 and 100 μg/ml to a tube containing control serum. After incubation at 37°C for 1 hr, the serum was placed in a micropartition tube (Centrifree TM, Amicon Co., Ltd., U.S.A.) and ultrafiltered by centrifugation at 3,500 r.p.m. for 30 min. The concentrations of SDMX in both the serum and ultrafiltrate were measured to calculate the binding rate of the drug to the serum proteins. The albumin concentration in the serum was analyzed by the bromcreosol green method [3].

Pharmacokinetic analysis: Pharmacokinetic parameters of SDMX in the skin and plasma obtained from the sacrificed animals were calculated by the Damping Gauss-Newton method [9]. The simulation curves of SDMX in the plasma obtained within 48 hrs after single intravenous administration were analyzed by the nonlinear perpendicular least-squares method [10].

Statistical analysis: Parallelism between the SDMX concentration-time curves in the skin and plasma during multiple injections was examined by the Parallel Line Assay [4].

RESULTS

The decay curve of SDMX in broiler skin after intravenous injection at a single dose of 200 mg/kg showed 2 phases (Fig. 1). The curve was fitted to a two compartment model with the following equation:

\[ C = 2.909e^{-0.1559t} + 0.124e^{-0.004003t}, \]

C: SDMX concentration in skin, t:hours after injection. The \( T_{1/2} \) was calculated to be 4.4 hrs in the 1st elimination phase and 173 hrs in the 2nd elimination phase (Table 2). The SDMX concentrations at 24 hrs after the injection were calculated to be 69.0 μg/g in the

![Fig. 1. Residual concentrations of SDMX in skin and plasma after single intravenous injection. ○, △, ■: skin, ○, Δ, □: plasma. Dose rate, circle: 30 mg/kg, triangle: 100 mg/kg, square: 200 mg/kg. Values are shown as mean ± S.D. (n=3). Dot line: detection limit. Arrows with dot line show no detection in next point.](image)
Table 2. T\textsubscript{1/2} of SDMX in broiler skin and plasma after single intravenous injection

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Skin</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>100</td>
<td>5.7</td>
<td>2.3</td>
</tr>
<tr>
<td>200</td>
<td>4.4\textsuperscript{a}</td>
<td>173\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} T\textsubscript{1/2} was calculated using the mean values at each sacrifice point (Fig. 1).
\textsuperscript{b} First elimination phase.
\textsuperscript{c} Second elimination phase.

1st elimination phase and 0.11 μg/g in the 2nd one. On the other hand, the curves of SDMX in skin after injection at the lower doses of 30 and 100 mg/kg were fitted to a one compartment model with T\textsubscript{1/2} of 3.2 and 5.7 hrs, respectively. All of the decay curves of SDMX in plasma declined linearly (Fig. 1) with T\textsubscript{1/2} of 2.3–4.3 hrs (Table 2).

Fig. 2. Comparison of SDMX concentrations in skin and plasma during multiple injections. ●, ○: method 1; ▲, Δ: method 2; ■, □: method 3 (Table 1). Closed and open marks represent the mean ±1 S.D. (n=3) calculated in skin and plasma, respectively.

The SDMX concentrations in the skin and plasma of the broilers injected repeatedly using method 1 or 2 (Table 1) increased after every injection (Fig. 2). On the other hand, the concentrations determined during injections using method 3 showed a steady state. In all cases, the concentration-time curve of SDMX in the skin was significantly (P<0.05) parallel with that in the plasma (Fig. 2). The SDMX concentrations in the skin were almost half of the corresponding concentrations in the plasma.

The decay curves of SDMX in the plasma obtained during the first 48 hrs after single intravenous injection at a dose more than 100 mg/kg showed two phases (Fig. 3). The curves were fitted to a nonlinear model with V\text{max} of 16.0 μg/ml/hr, Km of 72.4 μg/ml and V\text{d} of 0.508 l/kg. The SDMX concentration in the plasma was greater than 100 μg/ml for 12 to 30 hrs after the injection of 100 and 200 mg/kg.

The mean binding rates (n=3) of SDMX to serum protein were 76, 85 and 64% at a concentration of 1, 10 and 100 μg SDMX/ml, respectively. The mean albumin concentration in the control serum (n=3) was 1.60 g/dl.

The SDMX administered to the broilers via their drinking water at a concentration of 500 ppm for 5
days was not detected in either the plasma or any other edible tissue except the skin 3 days after withdrawal. On the other hand, the SDMX residue in the skin was observed up to 5 days after withdrawal (Fig. 4).

DISCUSSION

The decay curve of SDMX in broiler skin after single intravenous injection at a dose of 200 mg/kg was two phasic and fitted to the two compartment model (Fig. 1). The $T_{1/2}$ of the 2nd elimination phase was about 40 times greater than that of the 1st elimination phase (Table 2). This slow elimination might cause the long residue of SDMX in the broiler skins after administration via drinking water as reported in the previous paper [7]. The extrapolated SDMX concentrations in the skin at 24 hrs after injection at the high dose were calculated to be 69.0 μg/g in the 1st elimination phase and 0.11 μg/g in the 2nd one. The level of SDMX in the 1st elimination phase was comparable to the plasma level, which was undetectable during the 2nd elimination phase. Postulation of existence of two peripheral compartments for SDMX in the skin (Fig. 5) explained these results. The concentration in the 1st compartment ran parallel with that in plasma and only a small portion of plasma SDMX entered into the 2nd compartment from which eliminated very slowly.

In all cases of multiple intravenous injections, the concentration-time curve of SDMX in skin was parallel to that in plasma (Fig. 2). Therefore, the transfer of SDMX between a central compartment and the 1st peripheral compartment might be easy. The easy transfer was also verified by the Vd value of 0.508 l/kg calculated in this study. On the other hand, the transfer from the central compartment to the 2nd peripheral compartment might be difficult since an accumulation of SDMX in skin was not observed during the multiple injections.

A portion of a drug not bound to proteins in plasma can only pass into interstitial fluid [1, 2, 8]. The mean serum protein binding ratio of SDMX in the range from 1 to 100 μg/ml was 64-85%. Therefore, more than 15% of SDMX in plasma might be transferred into interstitial fluid in skin.

The slope of the decay curve in plasma during the first 48 hrs after single injection became smaller by increasing the dose resulting in nonlinear kinetics (Fig. 3). The nonlinear kinetics of SDMX in broiler plasma has been reported to be dependent on the saturation of the elimination of its metabolite [5, 6]. The kinetics produced a plasma concentration over 100 μg/ml for 12 to 30 hrs after injection at a dose of 100 and 200 mg/kg (Fig. 3). The sustained high plasma concentration might be responsible for a greater residue in skin. The nonlinear kinetics in plasma was not observed after single intravenous injection (Fig. 1). The initial time of the measurement in the study might be too late to find the nonlinear kinetics. $T_{1/2}$ of SDMX in plasma after single intravenous injection of 100 mg/kg was shorter than that after injection of 30 mg/kg (Table 2). The reason is not clear and further experiments should be needed.

The detectable residue period of SDMX in the skin after administration through drinking water at a concentration of 500 ppm was approximately one third of that observed in the residue study [7] after administration at a concentration of 1,000 ppm (Fig. 4), while the daily intake amount of SDMX (92±7 mg/kg body weight, mean ± S.D., n=15) was one half of that (188±30 mg/kg, n=21) in the study at a concentration of 1,000 ppm. The difference in the residue period might be related to the SDMX level in plasma.

All these data seem to support the two compartment hypothesis for SDMX in broiler skin. In practice, SDMX in drinking water should be administered at a concentration below 500 ppm to avoid a long withdrawal period. The mechanism of the slow elimination of SDMX from the 2nd peripheral compartment is under investigation.

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REFERENCES


