Pharmacokinetics and Metabolism of Toltrazuril and Its Major Metabolites after Oral Administration in Broilers

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Toltrazuril is a symmetrical tiazinetrione compound. It is active against all intracellular developmental stages including those of schizogony and gametogony. In this study the disposition kinetics of toltrazuril (TZR) and its major metabolites (TZR-SO and TZR-SO2) in broiler chickens after single oral administrations of 10 or 20 mg/kg were investigated. Mean plasma concentrations of TZR peaked at 16.4 and 25.2 $\mu$g/mL at 5.0 and 4.7 h after dosing, respectively. TZR was converted to the short-lived intermediary metabolite toltrazuril sulfoxide (TZR-SO), and then further metabolized to the reactive toltrazuril sulfone (TZR-SO2) being actually more slow eliminated with 80.3 and 82.9 h than TZR (10.6 and 10.7 h) or TZR-SO (14.8 and 15.3 h) in low and high dosing groups, respectively. Prolonged elimination half-life of TZR-SO2 could be interpreted as the persistent clinical efficacy of TZR in the treatment of protozoal parasites infection.

Key words: broiler, pharmacokinetics, toltrazuril, toltrazuril sulfone, toltrazuril sulfoxide


Introduction

Coccidiosis is a particularly dangerous disease in the poultry industry where chickens are maintained on the floor (Greif, 2000). Anticoccidial drugs of many different chemical types have been the dominant means to prevent and control the coccidiosis (Polozowski, 1993; Greif et al., 2001). However, the massive and uncontrolled use of anticoccidial drugs has resulted in the emergence of drug-resistant parasites (Suo et al., 2006).

Toltrazuril (TZR), 1-methyl-3-[3-methy]-4-[4-(trifluoromethylsulfinyl) phenoxy] phenyl]-1, 3, 5-triazinan-2-4, 6-trione, is a symmetrical triazinetrione compound. It has cocidoidal action against all intracellular developmental stages including those of schizogony and gametogony (Mehlhorn et al., 1984; Haberkorn and Stoltefuss, 1987). It has been reported to be effective against all coccidial species in most animal species, such as chickens (Mehlhorn et al., 1984), ducks (Chauve et al., 1994), dogs (Daugschies et al., 2000), mice (Haberkorn et al., 1983), pigeons (Van Reeth and Vercruysse, 1993), piglets (Mundt et al., 2007) and rabbits (Peeters and Geeroms, 1986).

TZR undergoes extensive metabolism to toltrazuril sulfoxide (TZR-SO) and then to TZR-SO2, which appears to have anticoccidial activity (Benoit et al., 1993; Lang et al., 1996; Bach et al., 2003; Mundt et al., 2007) (Fig. 1). Generally, triazines produced the same types of metabolites in most species, but the ratios of the metabolites showed the species differences (Furr and Kennedy, 2000; MacKay, 2006; Kim et al., 2010; Lim et al., 2010). Also, TZR is almost exclusively metabolized to TZR-SO2, also known as ponazuril (PZR), which has been recognized as the marker metabolite in a number of tissues. Although TZR is commonly used for the prevention and treatment of coccidiosis in broilers, the pharmacokinetic and metabolic profiles of TZR in broilers have received minimal investigation. There must be assessed not only in terms of good clinical efficacy but also considering the pharmacokinetics and metabolism of TZR in broilers for the rational therapeutic use of TZR in poultry. In this study, the aim of this study was to evaluate pharmacoki-
The pharmacokinetic parameters of TZR, TZR-SO and TZR-SO₂ were calculated by the non-compartmental analysis using the WinNonlin 6.1 (Pharsight Corporation, NC, USA). Peak plasma concentrations (Cₘₐₓ) of the drug and times to reach the peak concentration (tₘₐₓ) were determined from the individual plasma concentration-time curves. Lambda z (λ₂) is a first-order rate constant associated with the terminal (log linear) segment of the curve. It was estimated by the linear regression of the terminal data points. The terminal elimination half-life (t₁/₂λ₂) was calculated by t₁/₂λ₂ = 0.693/λ₂. The area under the plasma concentration-time curves (AUC) was calculated by the method of trapezoids. The area under the first moment curve (AUMC) was calculated as the product of time and drug concentration-time. Mean residence time (MRT) was calculated from MRT = AUC/AUMC. Apparent clearance (CL/F) from plasma was calculated from CL/F = dose/AUC and apparent volume of distribution (Vd/F) was calculated using Vd/F = CL/λ₂.

Results and Discussions

Prudent use of highly potent anticoccidials, such as TZR, in veterinary medicine is strongly required to maintain the efficacy and safety of TZR for the future. Therefore, its plasma disposition characteristics should be considered in choosing dosage regimens that maximize efficacy and minimize development of drug resistance.

No adverse effects of the treatment were observed in the two groups throughout the experimental period. TZR, TZR-SO and TZR-SO₂ were detected after oral treatment with TZR at two different dosage levels in broilers.

The mean plasma concentration profiles of TZR, TZR-SO and TZR-SO₂ obtained after oral administration of TZR in broilers are shown in Fig. 2. The results of the pharmacokinetic analysis for TZR and its metabolites after TZR treatments are summarized in Table 1. Peak plasma concentra-
tion (Cmax) and AUC value were shown to be in the dose dependent manners after oral administration of TZR at 10 or 20 mg/kg (Table 1).

The absorption of chemicals from the gastrointestinal tract depends on physiochemical properties of compounds, such as lipid solubility, and dissociation rate (Houston et al., 1974). Triazine-based antiprotozoal agents are known for their lipophilic characteristics and expected to be well absorbed following oral administration (Dirikolu et al., 2009). In the present study, the mean Cmax of TZR in broilers calculated in the present study were 16.4 μg/mL at 5.0 h after oral administration of 10 mg/kg of TZR. The apparent terminal elimination half-lives of TZR in broilers were 12.1 and 13.0 h after oral administration of 10 or 20 mg/kg of TZR, respectively. However, the half-life of TZR from our study was shorter than that in rats (23.0 h for 20 mg/kg, EMEA, 1998), rabbits (52.7 h for 10 mg/kg, 56.7 h for 20 mg/kg, Kim et al., 2010), pigs (48.7 h for 10 mg/kg, 68.9 h for 20 mg/kg, Lim et al., 2010), horses (55 h for 10 mg/kg, Tobin et al., 1997; 61.4 h for 10 mg/kg, Furr and Kennedy, 2000) and calves (154.0 h for 15 mg/kg, EMEA, 2004). These clearly indicate species-specific differences in terms of absorption and elimination characteristics of TZR following oral dosing.

Orally dosed TZR in mammals are absorbed from the gastrointestinal tract and are then subjected to a first-pass metabolism where the parent compound is rapidly converted to the active TZR-SO2 via a short-lived intermediary metabolite, TZR-SO. In broilers, TZR also rapidly metabolized to TZR-SO and TZR-SO2, which were detected as main metabolites of TZR in plasma. The mean Cmax of TZR-SO and TZR-SO2 in broilers were 8.2 μg/mL at 8.0 h and 17.0 μg/mL at 54.0 h following oral administration of TZR at 10 mg/kg. Maximum plasma concentration of TZR-SO was observed very early, suggesting the conversion of the parent drug to TZR-SO was very rapid following oral administration of TZR. After oral administration of 10 mg/kg of TZR, elimination half-lives of TZR-SO in broiler was 14.7 h, much shorter than in rabbits (56.1 h, 10 mg/kg of TZR, Kim et al., 2010) and pigs (51.9 h, 10 mg/kg of TZR, Lim et al., 2010). The average plasma elimination half-life of TZR-SO2 in broilers has been reported to be around 80.3 h after oral administration of TZR (10 mg/kg), being similar to that observed in rabbits (Kim et al., 2010).

Fig. 1. Plasma concentration-time curves of TZR (●), TZR-SO (○) and TZR-SO2 (▼) after oral administration of TZR at 10 mg/kg (A, n = 6) or 20 mg/kg (B, n = 6) in broilers. Data were shown as mean ± SD.

The physiological pH in the avian stomach is low, particularly at the gizzard which is considered as a powerful triturating machine and facilitates the disintegration of solid oral dosage forms (Vermeulen et al., 2002). These properties could contribute to enhance TZR solubility and its absorption processes in broilers compared to other species. Furthermore, the greater tendency towards high first-pass loss in birds were reported since gastric and intestinal emptying time hardly influence the absorption of liquid dosage forms of readily soluble drugs (Dorrestein, 1992; Neirinckx et al., 2010). In the present study, TZR was shown relatively higher Cmax at earlier Tmax in broilers as compared to other mammalian species. In addition, shorter half-lives of TZR and TZR-SO in broilers than other species possibly owing to extensive first-pass metabolisms were also observed. These interspecies differences in TZR absorption and metabolism are a major factor accounting for its species differences in pharmacokinetics, efficacy and registration scheme. Especially, a maximum residue limit (MRL) for TZR-SO2 of 0.1 ppm in muscle tissue, 0.2 ppm in the skin+fat, 0.6 ppm in the liver and 0.4 ppm in the kidney tissue has been established (EMEA, 2004). Depletion of TZR-SO2 from animal tissues is known to be relatively slow. TZR-SO2 has a broad spectrum anticoccidial activity (Franklin et al., 2003; Gottstein et al., 2001; Lindsay et al., 2000). TZR-SO2 also affects the intracellular developmental stages of protozoan parasites, leading to their swollen mitochondria and enlarged endoplasmic reticulum (Mehlhorn et al., 1984). The TZR-SO2 should be regarded as a suitable marker residue in broilers based on this study.

There is little information in the literature about the in vivo and in vitro sensitivity of Eimeria tenella (E. tenella) and Neospora caninum (N. caninum) to TZR and its major me-
Pfefferkorn, 1993), TZR-SO2 also has a potent and broad spectrum anticoccidial activity (Lindsay et al., 2001; Franklin et al., 2003). In one of the recent studies, TZR at 10 μg/mL and TZR-SO2 at 30 μg/mL also led to a significant reduction in the multiplication rate of *N. caninum* (Darius et al., 2004). Mitchell et al. (2005) reported that TZR-SO2 at 5 μg/mL inhibited development of *N. caninum* after approximately 48 h post exposure in African green monkey kidney cell culture. Prolonged elimination half-life of TZR-SO2 in broilers could be interpreted as the persistent clinical efficacy of TZR in the treatment of protozoal parasites infection.

In conclusion, TRZ was absorbed very well through the gastrointestinal tract and metabolized to TZR-SO and TZR-SO2 after oral administration of TZR in broilers. TZR-SO2, an equi-efficacious metabolite, was actually more slowly eliminated than TZR and TZR-SO. These results translate into the clinical efficacy of TZR, and give valuable information about the metabolism and elimination of TZR in broiler chickens.

### Acknowledgments

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### References


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Benoit E, Buronfosse T, Moroni P, Delatour P and Riviere JL. Stereoselective S-oxygenation of an aryltrifluoromethyl sulfoxide to the corresponding sulfone by rat liver cytochromes P450. Stereoselective S-oxygenation of an aryltrifluoromethyl sulfoxide to the corresponding sulfone.


### Table 1. Pharmacokinetic parameters (*n*=6) of TZR, TZR-SO and TZR-SO2 in broilers after the oral administration of TZR (10 or 20 mg/kg)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Pharmacokinetic parameters †</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
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<tbody>
<tr>
<td>TZR</td>
<td><em>t</em>&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>5.0 ± 1.2</td>
<td>4.7 ± 1.0</td>
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<td></td>
<td><em>C</em>&lt;sub&gt;max&lt;/sub&gt; (μg/mL)</td>
<td>16.4 ± 2.0</td>
<td>25.2 ± 1.5</td>
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<tr>
<td></td>
<td>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (mg·h/mL)</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
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<td></td>
<td><em>t</em>&lt;sub&gt;1/2α&lt;/sub&gt; (h)</td>
<td>10.6 ± 1.2</td>
<td>10.7 ± 1.6</td>
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<td></td>
<td><em>V</em>&lt;sub&gt;α&lt;/sub&gt;F (mL/kg)</td>
<td>0.7 ± 0.2</td>
<td>0.9 ± 0.2</td>
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<tr>
<td></td>
<td>CI/F (mL/kg/h)</td>
<td>40.4 ± 12.1</td>
<td>52.6 ± 12.2</td>
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<td></td>
<td>MRT (h)</td>
<td>15.0 ± 2.6</td>
<td>14.7 ± 2.7</td>
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<td>TZR-SO</td>
<td><em>t</em>&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>8.0 ± 2.8</td>
<td>7.5 ± 3.0</td>
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<tr>
<td></td>
<td><em>C</em>&lt;sub&gt;max&lt;/sub&gt; (μg/mL)</td>
<td>8.2 ± 1.0</td>
<td>11.8 ± 1.3</td>
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<tr>
<td></td>
<td>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (μg·h/mL)</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.0</td>
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<td><em>t</em>&lt;sub&gt;1/2α&lt;/sub&gt; (h)</td>
<td>14.7 ± 2.9</td>
<td>15.3 ± 2.6</td>
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<td></td>
<td>MRT (h)</td>
<td>21.8 ± 1.9</td>
<td>23.2 ± 2.1</td>
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<td>TZR-SO2</td>
<td><em>t</em>&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>54.0 ± 15.5</td>
<td>51.0 ± 20.5</td>
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<tr>
<td></td>
<td><em>C</em>&lt;sub&gt;max&lt;/sub&gt; (μg/mL)</td>
<td>17.0 ± 3.4</td>
<td>26.7 ± 3.1</td>
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<td></td>
<td>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (μg·h/mL)</td>
<td>3.3 ± 0.8</td>
<td>4.6 ± 0.8</td>
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<tr>
<td></td>
<td><em>t</em>&lt;sub&gt;1/2α&lt;/sub&gt; (h)</td>
<td>80.3 ± 10.8</td>
<td>82.9 ± 9.3</td>
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<td></td>
<td>MRT (h)</td>
<td>125.3 ± 16.5</td>
<td>126.4 ± 6.6</td>
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</table>

Data were shown as mean ± SD.

† *t*<sub>max</sub>, time of maximum observed concentration; *C*<sub>max</sub>, maximum observed concentration; AUC<sub>0→∞</sub>, area under curve from 0 h to infinity; *t*<sub>1/2α</sub>, terminal elimination half-life; *V*<sub>α</sub>F, apparent volume of distribution; CI/F, apparent clearance; MRT, mean residence time.
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