Pharmacokinetics of Toltrazuril and Its Metabolites, Toltrazuril Sulfoxide and Toltrazuril Sulfone, after a Single Oral Administration to Pigs

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(Received 19 November 2009/Accepted 9 March 2010/Published online in J-STAGE 24 March 2010)

ABSTRACT. Toltrazuril (TZR) is a triazine-based antiprotozoal agent. Following a single oral administration of TZR at 10 and 20 mg/kg to male pigs, the mean TZR concentration in plasma peaked at 4.24 and 8.18 $\mu\text{g/ml}$ at 15.0 and 12.0 hr post-dose, respectively. TZR absorbed was rapidly converted to the short-lived intermediary metabolite toltrazuril sulfoxide (TZR-SO), and then metabolized to the reactive toltrazuril sulfone (TZR-SO₂). TZR-SO₂ was actually more slowly eliminated, with average half-lives of 231 and 245 hr, compared with TZR (48.7 and 68.9 hr) or TZR-SO (51.9 and 53.2 hr) in the 10 and 20 mg/kg groups, respectively. This study demonstrates that TZR metabolizes to TZR-SO₂ having a long-terminal half-life, enabling the persistent clinical efficacy in the treatment of *I. suis* infection. In contrast, special consideration should be given to the residual of TZR-SO₂.

KEY WORDS: pharmacokinetics, toltrazuril, toltrazuril sulfone, toltrazuril sulfoxide.

J. Vet. Med. Sci. 72(8): 1085–1087, 2010

Isospora suis is one of the most prevalent parasites in intensive pig production causing significant economic losses due to transient diarrhea in young piglets with subsequently decreased weight gain and poor performance [12, 14, 15].

Toltrazuril [TZR, 1-methyl-3-{3-methyl-4-[4-(trifluoromethylthio) phenoxy]phenyl}-1,3,5-triazine-2,4,6 (1H, 3H,5H)-trione], is a triazine-based antiprotozoal agent [13]. It has broad-spectrum anticoccidial and antiprotozoal activity. It is widely used in chickens, turkeys, pigs and cattle for prevention and treatment of coccidiosis, by administration via drinking water [7, 8, 12–14]. TZR 5% oral solution (Baycox[®] 5%, Bayer HealthCare, Animal Health, Berlin, Germany) is the only anticoccidial registered for the control of porcine neonatal coccidiosis.

TZR is similar to but different from benzoimidazoles in their metabolic profiles. Benzoimidazoles are rapidly oxidized to sulfoxide metabolites responsible for the anthelmintic activity, which undergo bioconversion to pharmacologically inactive sulfone metabolites [11]. On the other hand, TZR undergoes extensive metabolism to toltrazuril sulfoxide (TZR-SO) and then to toltrazuril sulfone (TZR-SO₂), which appears to have anticoccidial activity [1, 2, 10, 14]. Generally, triazines produced the same types of metabolites in most species, but the ratios of the metabolites showed the species differences [10]. However, the pharmacokinetic and metabolic profiles of TZR in pig were not fully elucidated. In this study, the pharmacokinetics of TZR and its major metabolites were evaluated after a single oral

administration of 10 or 20 mg/kg to pigs.

TZR was obtained from Riedel de Haen (Seelze, Germany) and TZR-SO, TZR-SO₂ and nicarbazin (internal standard, IS) were purchased from Sigma (St. Louis, MO, U.S.A.). HPLC grade methanol and acetonitrile were purchased from Mallinckrodt Baker (Phillipsburg, NJ, U.S.A.). Other analytical grade chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

A single dose of 10 or 20 mg/kg of TZR solution (Baycox[®] 5% suspension, Bayer Korea, Asan, South Korea) was orally administered to six male pigs (Landrace $\times$ Yorkshire $\times$ Duroc, 4-month-old, 39.7 ± 4.68 kg), respectively. Blood samples were obtained by jugular vein puncture at different time points (0, 0.5, 1, 2, 4, 6, 8 and 12 hr and 1, 2, 3, 6, 9, 18 and 36 days) after the oral administration of TZR. The samples were centrifuged at 1,500 g for 10 min to obtain plasma and stored at -70°C until analysis. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Chungnam National University.

Samples were analyzed on an Agilent 1100 series LC/MSD system. Separation was achieved on a C₁₈ reverse phase column (Eclipse plus[®], 3.5 μm , 4.6×100 mm, Agilent, Santa Clara, CA, U.S.A.). TZR and its metabolites were eluted by isocratic runs with a mixture of deionized water-acetonitrile (30:70, v/v). The compounds were eluted at ambient temperature at a flow rate of 0.6 ml/min. Mass spectrometry was performed using negative ion mode and the selected ion monitoring (SIM), detecting TZR at m/z 424.0, TZR-SO at m/z 440.0, TZR-SO₂ at m/z 456.0 and nicarbazin (IS) at m/z 301.0 with a dwell time of 300 msec.

The pharmacokinetic parameters of TZR, TZR-SO and TZR-SO₂ were calculated by non-compartmental analysis using WinNonlin 5.2 (Pharsight, Mountain View, CA,

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U.S.A.). Peak plasma concentrations ($C_{\max}$) of the drug and times to reach the peak concentration ($t_{\max}$) were determined from the individual plasma concentration-time curves. Lambda z (λ_z) 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 ($t_{1/2\lambda_z}$) was calculated by $t_{1/2\lambda_z} = 0.693/\lambda_z$. 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 $MRT = AUC/AUMC$.

Comparison of the chromatograms of blank and spiked swine plasma samples indicated no significant interferences at the retention times of analytes and IS. The peaks of TZR, TZR-SO, TZR-SO₂ and IS were observed at 5.6, 3.3, 4.0 and 4.6 min, respectively. The peak area ratios of TZR, TZR-SO and TZR-SO₂ to IS were used for construction of the calibration curves, ranged from 0.05 to 10 $\mu\text{g/ml}$, and the standard curves were fitted to linear regression. The calibration curve was shown to have good linearity ($r^2 > 0.99$) over the range of 0.05–10 $\mu\text{g/ml}$. Interassay precision values were less than 13.36%, and the accuracy values ranged from 93.09 to 116.2% for five replicate quality control (QC) samples at the concentration levels of 0.05, 0.25, 2.5 and 8 $\mu\text{g/ml}$. The intra-assay precision values were less than 12.5%, and the accuracy values ranged from 96.4 to 118.4% for the QC samples. These precision and accuracy values indicated that the assay method was consistent and reliable. The LC/MS analysis for the determination of TZR, TZR-SO and TZR-SO₂ could be performed rapidly with a high degree of reproducibility and was successfully applied to the pharmacokinetic study.

No side effects, such as nausea, vomiting, abdominal cramps and diarrhea, associated with single oral administration of TZR at a dose of 10 or 20 mg/kg of bodyweight in the pigs were found. The mean TZR, TZR-SO and TZR-SO₂ plasma concentration profile and pharmacokinetic parameters based on non-compartmental analysis for the pigs are shown in Fig. 1 and Table 1.

TZR was well absorbed through the gastrointestinal tract with mean $C_{\max}$ values of 4.24 and 8.18 $\mu\text{g/ml}$ at 15.0 and

Fig. 1. Plasma concentrations of TZR (●), TZR-SO (○) and TZR-SO₂ (▼) after a single oral administration of TZR (10 mg/kg) to pigs. The data are shown as means $\pm$ SD.

12.0 hr after administration of an oral dose of 10 or 20 mg/kg of TZR to the pigs, respectively. The absorption of TZR was increased with dosage following oral administration of TZR at 10 or 20 mg/kg to the pigs. The mean maximal plasma concentration in this study was similar to those of studies in which TZR was administered at an oral dose (10 mg/kg) yield $C_{\max}$ values of 4.50–11.7 $\mu\text{g/ml}$ at 18 hr in horses [6, 16].

Doses of TZR 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. The peak plasma concentration of TZR-SO was observed very early (mean $C_{\max}$ values of 1.91 and 4.01 $\mu\text{g/ml}$; mean $t_{\max}$ of 21.0 and 24.0 hr) suggesting that the conversion of the parent drug to TZR-SO was very rapid following oral administration of TZR at 10 or 20 mg/kg, respectively. However, the additional oxidative step is slower, and a marked delay of the peak plasma concentration of TZR-SO₂ (mean $C_{\max}$ values of 4.12 and 8.74 $\mu\text{g/ml}$; mean $t_{\max}$ of 108 and 126 hr) in the pigs was shown with a single oral administration of TZR at 10 or 20 mg/kg, respectively. In the case

Table 1. Main pharmacokinetic parameters of TZR, TZR-SO and TZR-SO₂ after a single oral administration of TZR (10 or 20 mg/kg) to pigs

Pharmacokinetic parameters [†] (unit)	TZR		TZR-SO		TZR-SO ₂	
	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg
$t_{\max}$ (hr)	15.0 $\pm$ 6.0	12.0 $\pm$ 0.0	21.0 $\pm$ 6.0	24.0 $\pm$ 0.0	108 $\pm$ 41.6	126 $\pm$ 36.0
$C_{\max}$ ($\mu\text{g/ml}$)	4.24 $\pm$ 0.97	8.18 $\pm$ 0.95	1.91 $\pm$ 0.29	4.01 $\pm$ 1.24	4.12 $\pm$ 1.60	8.74 $\pm$ 3.40
$t_{1/2\lambda_z}$ (hr)	48.7 $\pm$ 10.2	68.9 $\pm$ 1.94	51.9 $\pm$ 1.04	53.2 $\pm$ 2.14	231 $\pm$ 58.6	245 $\pm$ 56.2
AUC _{0–∞} (mg·hr/ml)	0.39 $\pm$ 0.13	0.68 $\pm$ 0.08	0.20 $\pm$ 0.02	0.48 $\pm$ 0.026	1.96 $\pm$ 1.03	4.20 $\pm$ 2.23
MRT (hr)	77.2 $\pm$ 13.4	96.2 $\pm$ 1.23	84.2 $\pm$ 9.62	84.3 $\pm$ 10.6	372 $\pm$ 77.7	380 $\pm$ 79.3

*Data are shown as means $\pm$ standard deviation (SD).

[†] $t_{\max}$, time of maximum observed concentration; $C_{\max}$, maximum observed concentration; $t_{1/2\lambda_z}$, terminal half-life; AUC_{0–∞}, area under curve from 0 hr to infinity; MRT, mean residence time.

of single oral administration of TZR-SO₂ as the parent drug, the mean peak plasma concentration of TZR SO₂ was shown to be 4.56 µg/ml at 57.4 hr following a single 5 mg/kg oral dose of TZR SO₂ in cows [4] and 2.40 µg/ml at 29.04 hr following a single oral dosing of 2.2 mg/kg of TZR SO₂ in horses [5].

Plasma pharmacokinetic studies in association with studies of the efficacy of antiparasitics against parasites remain the main tool to evaluate the efficacy of antiparasitics and avoid their misuse, which leads to widespread distribution of parasite resistance. The anticoccidial activity of TZR can be attributed to the systemic sum of the parent compound and the TZR-SO₂ because TZR and TZR-SO₂ have previously been revealed to have excellent efficacy against *I. suis* in piglets, affecting all endogenous parasite stages [1, 14]. In the present study, TZR-SO₂ after oral administration of TZR at 10 mg/kg in the pigs was more slowly eliminated than TZR and TZR-SO₂, their mean half-lives were 231, 48.7 and 51.9 hr, respectively. The prolonged elimination half-life of TZR-SO₂ was consistent with the persistent clinical efficacy of TZR in the treatment of *I. suis* infection [13, 14]. Therefore, the TZR-SO₂ should be regarded as a suitable marker residue in pigs based on this study. In the European Union (EU), maximum tolerated residue levels (MRL) of TZR have also been proposed for edible pig tissues (0.1 ppm for the muscle, 0.15 ppm for the skin + fat, 0.5 ppm for the liver and 0.25 ppm for the kidney as TZR-SO₂, marker residue [3]). Further study is needed on TZR depletion and metabolism in edible tissues in order to establish appropriate withdrawal periods in pigs.

In conclusion, this study demonstrates that TZR metabolizes to its active metabolite, TZR-SO₂, which has a long terminal half-life in excess of 200 hr. These results contribute to interpreting the clinical efficacy of TZR and provide valuable information about the metabolism and elimination of TZR in pigs. In particular, given the fact that the MRL for muscle is 0.1 ppm, special consideration should be given to the residual TZR-SO₂ because the plasma concentration remained around 1 ppm at even about 900 hr (ca. 38 days) after a single oral treatment of TZR at 10 mg/kg.

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