

Comparative Pharmacokinetic of Three Sulfadiazine Suspensions by Oral Administration in Chickens

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Abstract

The chemotherapeutics, sulfadiazine (SDA) and trimethoprim (TMP), are extensively used in a variety of animal species. In this study, a pharmacokinetic analysis was performed to compare the bioequivalence of a combined SDA and TMP product against existing licensed SDA and TMP formulations in broiler chickens. Three groups of 15 birds were administered a single dose of either the test formulation or a reference oral suspension. The plasma concentration of SDA and TMP were determined by reverse-phase high performance liquid chromatography (HPLC), and the maximal plasma concentration (C_{max}), area under the curve (AUC), the peak time (T_{max}), mean residence time (MRT) and elimination half-life ($T_{1/2}$), were calculated for SDA. The combined formulation I and II reference suspension exhibited almost identical concentration-time curves, and ANOVA analyses of the pharmacokinetic parameters identified no significant differences between the reference preparations and the test one. Furthermore the AUC and C_{max} values of the SDA active ingredient were not significantly different. The I formulation was bioequivalent with both II and III (80-125% and 70-143%, respectively, at the 90% confidence interval). In conclusion, the combined SDA and TMP product was bioequivalent with both existing commercially available SDA suspensions and can be used interchangeably in veterinary medical practice.

Keywords: sulfadiazine, trimethoprim, HPLC, oral administration, pharmacokinetics

1. Introduction

Classical combinations of sulfadiazine (SDA) and trimethoprim (TMP) have been used extensively to treat serious infections of bacterial or protozoal origin in a range of animal species for over 35 years, with particular use in respiratory and alimentary tract infections (Bushby, 1980; Nielsen & Gyrd-Hansen, 1994; Ensink et al., 2003). Combining these two chemotherapeutics has a synergistic antibacterial effect *in vitro* caused by the inhibition of a different step in the bacterial folic acid biosynthetic pathway (Batziar et al., 2005). This synergism not only lowers the minimum inhibitory concentration (MIC) of both drugs, but also broadens the bacterial spectrum and decreases resistance occurrence (Bushby, 1980; Van Duijkeren et al., 1994; Plumb, 2002). In veterinary practice, SDA and TMP are generally used at a ratio of 5:1 (Riviere & Spoo, 2001; Batziar et al., 2005) and this has proved effective against a wide range of pathogenic bacteria (Emms et al., 1987; Rogers et al., 1988; Clarke et al., 1989; Gookin et al., 1999; Rothschild et al., 2004; Ensink et al., 2003, 2005).

The pharmacokinetic profile of SDA and TMP used together has been reported for chicken (Löscher et al., 1990; Batziar et al., 2000; Baert et al., 2003) and ostrich (Abu-Basha et al., 2009). Studies in other species such as swine (Søli et al., 1990; Nielsen & Gyrd-Hansen, 1994; Garwacki et al., 1996; Baert et al., 2001), cattle (Clarke et al., 1989), camel (Kumar et al., 1998), horse (Brown et al., 1983; Van Duijkeren et al., 1994), dog (Sigel et al., 1981), donkey (Oukessou et al., 1998), Japanese quail (Lashev et al., 1994) and carp (Nouws et al., 1993) have also been performed. All studies reported a higher volume of distribution (V_d) or clearance (Cl), and a substantially shorter $T_{1/2}$ for TMP compared with SDA.

Product I is a generic SDA/TMP combined oral suspension formulated to contain the same active constituents as the licensed original products II and III, and contains identical amounts of SDA and TMP in a ratio of 5:1. In

China, three combined SDA/TMP oral suspensions are recommended for use in broiler chickens to treat sensitive bacterial and protozoal diseases including *E. coli septicaemia*, *Salmonellosis* (including fowl typhoid and pullorum disease), and secondary bacterial infections associated with viral respiratory disease. Although both generic and original products contain the same active ingredient, they have different manufacturing processes and contain different excipients that can affect the rate and extent of absorption of the active molecule. To prevent the development of bacterial resistance against these agents, and generic preparations must be bioequivalent, and this has to be determined by a controlled pharmacokinetic study.

In general, if bioequivalence can be demonstrated, the generic product can be considered equivalent in terms of efficacy and safety for therapeutic use. However, changes in pharmacokinetic variables may modify withdrawal times and negatively impact the clinical outcome. In the present study, we sought to confirm the bioequivalence between a generic SDA product and existing licensed products following single-dose administration by measuring C_{max} , T_{max} and AUC in healthy broiler chickens.

2. Materials and Methods

2.1 Drugs and Reagents

The reference substances SDA (batch no. H0361105) and TMP (batch no. H0160704) were purchased from the China Institute of Veterinary Drug Control (Beijing, P. R. China). Three oral suspensions containing identical concentrations of SDA (400 mg/mL) and TMP (80 mg/mL) were compared in this study. The test preparation I (Chinanimal Nanjing Veterinary Drugs Co. Ltd., China) and the reference preparation (Virbac Animal Health Co. Ltd.) and III (Intervet/Schering-Plough Animal Health Co. Ltd.) were analyzed by HPLC to confirm the SDA and TMP content, which were found to be in agreement with the values given by the manufacturers.

Methanol and acetonitrile (Fisher, France) and all other reagents used in this study were analytically pure and HPLC grade unless stated otherwise.

2.2 Animals

A total of 45 male and female healthy White Plymouth Rock broilers at the age of 35 days were obtained from a local farm. The birds were housed in three groups of 15 and monitored for one week for clinical signs of disease before the experiment. The chickens had free access to antibacterial-free fodder and water.

All of the animal studies were approved by the Jiangsu Administrative Committee for Laboratory Animals (Permission number SYXKSU-2007-0005).

2.3 Study Design

Due to relatively rapid growth rate and body weight gain of broilers, a parallel study was carried out. Three groups of 15 chickens were given a single 24 mg/kg oral dose of either product I, II or III containing SDA and TMP (20 and 4 mg/kg, respectively) using a stomach tube. To minimize absorption variability, birds were fasted for 12 h before drug administration, and water was withdrawn 1 h before administration. Access to water was restored immediately after dosing and food was provided 2 h later.

Blood samples were collected from the left brachial vein into heparinized plastic tubes at 0 (before treatment), 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 16 and 24 h after drug administration. The blood volume was 1.5 ml each time. Samples were centrifuged at 3500 rpm for 5 min to obtain plasma and stored at -20 °C until needed for analysis.

2.4 Analytical Method

The concentration of SDA and TMP in plasma were determined by reverse-phase high performance liquid chromatography (HPLC) using ultraviolet (UV) detection. The chromatographic method was slightly modified from that described by Abu-Basha et al. (2009). After the addition of 2 mL acetonitrile to 500 μ L of plasma, samples were extracted by vortexing for 5 min and centrifuged for 15 min at 5000 rpm. The organic phase was transferred into a 50 mL pear-shaped flask, the procedure was repeated, and extracts were combined and evaporated to dryness using a rotary evaporator at 45 °C. Residues were re-dissolved in 1 mL mobile phase, transferred to a small tube and centrifuged for 15 min at 16000 rpm. The supernatant was filtered through a 0.22 μ m membrane, and 20 μ L was injected onto the HPLC column. Samples were analyzed within 16 h of preparation.

HPLC (Agilent, USA) was performed on a 250 mm \times 4.6 mm Hypersil ODS-2 5 μ m C_{18} column (Thermo Scientific, USA) with an appropriate guard column at room temperature. The mobile phase consisted of 14:86 acetonitrile:10 mM potassium dihydrogen phosphate that was filtered through a 0.45 μ m membrane and degassed. The mobile phase was eluted at a flow rate of 1.0 mL/min and progress was monitored using a UV

detector at a wavelength of 240 nm. The volume of injection was 20 μ L.

2.5 Validation

The method was validated prior to the analysis. Selectivity was confirmed as there were no interfering peaks from endogenous compounds with similar retention times to SDA and TMP. Elution times in plasma for SDA and TMP were 8.3 min and 11 min, respectively. Standard curves were linear between 0.06 mg/L and 10.0 mg/L ($r^2 > 0.999$) for SDA and 0.1–1.0 mg/L ($r^2 > 0.998$) for TMP. The mean percentage analytical recoveries in plasma and the intra-day and inter-day variations were investigated for both drugs (Table 1). The limit of detection (LOD) was the lowest concentration that could be determined by the UV detector with a signal-to-noise ratio of 3:1 and was 0.03 mg/L for SDA and 0.05 mg/L for TMP. The limit of quantification (LOQ) was the lowest concentration for which results fell within the ranges recommended by Jiyue and Xiaocong (2005) and were 0.06 mg/L for SDA and 0.1 mg/L for TMP. Both SDA and TMP were stable in chicken plasma following three freeze–thaw cycles, for 4 weeks after storage at -20 °C, for 24 h at 25 °C, and for 24 h after being processed. The coefficients of variation were all within 20% for stability tests, indicating that there was no significant degradation under described conditions.

Table 1. Recovery and measurement precision of SDA and TMP in broiler chicken plasma (mean \pm SD)

Drug	Concentration (mg/L)	Recovery (%)	Intra-day coefficient of variation (%)	Inter-day coefficient of variation (%)
SDA	0.06	123.02 \pm 2.90	0.11	1.99
	1	97.62 \pm 2.07	1.75	1.33
	10	100.24 \pm 0.73	0.65	2.37
TMP	0.1	112.37 \pm 2.24	0.11	1.70
	0.5	90.78 \pm 1.21	2.32	5.44
	1	97.08 \pm 2.30	1.11	1.78

2.6 Pharmacokinetic Analysis

Pharmacokinetic parameters of SDA and TMP were calculated from the concentration-time data using non-compartmental model. C_{\max} and T_{\max} were defined as the observed highest concentration and time of occurrence, respectively, and were obtained directly from the concentration-time curves. A non-compartmental analysis was carried out using the trapezoidal method to calculate the area under the curve (AUC). The elimination half-life ($T_{1/2}$) was calculated as $0.693/k$, where k is the negative gradient of the log-linear terminal portion of the plasma concentration-time profile determined using linear regression. Total body clearance (Cl) and mean residence time (MRT) were calculated using established methods (Baggot, 1977) with Kinetica software version 4.4 (Thermo Electron, USA).

2.7 Statistical Analysis

The pharmacokinetic parameters describing the rate and extent of drug absorption, C_{\max} , T_{\max} , AUC, $T_{1/2}$ and MRT were derived from the individual plasma concentration-time data and subjected to statistical analysis with SPSS 10.0. All values were expressed as mean \pm SD. ANOVA was used to estimate differences between test and reference formulations, and differences were considered significant when $P < 0.05$. After logarithmic transformation, AUC and C_{\max} were subjected to ANOVA.

For bioequivalence evaluation, 90% confidence intervals (CIs) were calculated for differences between test and reference formulations using the residual mean square error obtained from multifactorial ANOVA. For bioequivalence analysis, AUC and C_{\max} were considered primary variables, and bioequivalence was determined by calculating 90% CIs for the ratios of AUC and C_{\max} values for test and reference formulations using logarithmically transformed data. Formulations were considered bioequivalent if the 90% CI of AUC fell within the 0.8–1.25 range. The acceptance range for C_{\max} can be wider than that for AUC, especially for drugs of highly variable C_{\max} , and the recommended range is 0.7–1.43 if differences in T_{\max} are not statistically significant (FDA, 2012).

3. Results

3.1 Pharmacokinetic Characteristics

All chickens were clinically healthy throughout the study, and no adverse reactions were apparent after oral

administration of the drugs. Approximately 4-6 h after dosing, the TMP concentration was below or equal to the LOQ in all three groups, and TMP could not be detected in the plasma, whereas SDA was measurable even at the final sampling point (24 h) in all birds. The TMP was therefore not suitable for further pharmacokinetic analysis, and only plasma SDA was considered.

Mean plasma concentration-time profiles for SDA following a single oral dose of test or reference formulation were plotted (Figure 1) and primary pharmacokinetic parameters were determined (Table 2). These parameters showed similar mean values for all three groups, with only marginal differences between reference and test formulations. SDA was detected in plasma early and peak concentrations were reached at 3.87 h and 3.43 h with the reference formulations and at 4.93 h with the test product. Plasma SDA levels then decreased but were still detectable at 24 h. T_{max} values were also similar for all three preparations (Figure 1). Plasma concentration-time curves of the I test formulation and II reference formulation were extremely similar, whereas III had distinctly higher C_{max} and AUC values. Nevertheless, ANOVA showed that the mean values of C_{max} , $T_{1/2}$, MRT, AUC, and Cl were not significantly different between the test or reference groups.

Table 2. Pharmacokinetic parameters of SDA in broiler chickens following oral administration of test and reference formulations

Parameters	Tested preparation (I)	Reference preparation (II)	Reference preparation (III)
C_{max} (mg/L)	10.60 ± 6.68	11.45 ± 5.96	15.64 ± 5.22
T_{max} (h)	40.93 ± 2.25	30.87 ± 1.06	3.43 ± 1.74
$T_{1/2}$ (h)	40.72 ± 3.48	40.64 ± 1.75	3.95 ± 0.98
MRT (h)	70.82 ± 2.87	70.80 ± 2.09	6.62 ± 1.69
AUC (mg/L/h)	89.31 ± 48.50	86.81 ± 45.78	119.54 ± 39.93
Cl (L/kg/h)	70.94 ± 4.06	60.54 ± 2.70	3.23 ± 1.83

Note. Values are given as mean ± SD.

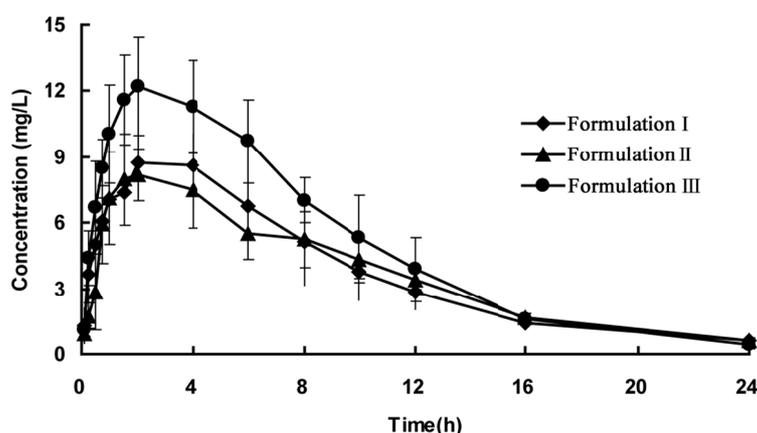


Figure 1. Mean plasma concentration-time curves of SDA in broiler chickens after oral administration of test and reference formulations

3.2 Bioequivalence Analysis

Bioequivalence analysis gave 90% CI values for I and II ratios (%) for AUC and C_{max} of 0.92–1.13 and 0.70–1.28, respectively. The 90% CI values for I and III ratios (%) for AUC and C_{max} were 0.90–1.08 and 0.72–1.02, respectively (Table 3). These CI values were within the bioequivalence range.

Table 3. Means and 90% confidence intervals of pharmacokinetic parameters of test (I) and reference (II) and (III) formulations

Pharmacokinetic parameter	I/II		I/III		Bioequivalence limit
	Mean	90% CI	Mean	90% CI	
	ratio		ratio		
Ratio of AUC	1.03	0.92–1.13	0.99	0.90–1.08	0.80–1.25
Ratio of C_{max}	0.99	0.70–1.28	0.87	0.72–1.02	0.70–1.43

4. Discussion

This study examined the pharmacokinetic properties and bioequivalence of three oral formulations of SDA in healthy broiler chickens. The concentration–time curves of the test formulation I and the reference formulation II almost overlapped (Figure 1), and mean AUC and C_{max} values of the active ingredient SDA were not significantly different. Furthermore, 90% CIs for both I vs. II and I vs. III confirmed that the test product was bioequivalent with the two commercially available SDA suspensions II and III.

There are only a few reports in the literature regarding the pharmacokinetics of SDA and TMP combination products in chickens, and the results are controversial (Löscher et al., 1990; Baert et al., 2003). Löscher et al. (1990) reported that SDA and TMP were rapidly eliminated from plasma following oral administration, with half-lives of 2.7 h and 1 h, respectively. In contrast Baert et al. (2003) reported mean half-lives of 3.2 h and 3.71 h following intravenous or oral administration of SDA (33.34 mg/kg), respectively. Others researchers reported different half-lives for SDA ranging from 1.13 h from Dagorn et al. (1991) to 2.8 h from Batzias et al. (2000). In the present study, the half-life values of SDA for all three preparations were higher than those reported previously by Löscher et al. (1990) and Baert et al. (2003). This extended half-life could be due to delayed absorption caused by the presence of food in the intestine or differences in oral dose administration. The extent of absorption is a key consideration for drug formulation, and AUC is an important parameter for comparative bioavailability or bioequivalence studies. C_{max} and T_{max} are also important parameters of the plasma profile, and could affect the therapeutic use of a drug.

Baert et al. (2003) reported mean values for SDA in broiler of 292.1 mg/h/L, 39.32 mg/L and 1.64 h for AUC, C_{max} and T_{max} , respectively. In the present study, AUC (86.81–119.54 mg/h/L) and C_{max} (10.6–15.64 mg/L) for SDA were significantly lower. This difference could be due to differences in the breed or differences in the oral dose administered (33.34 mg/kg vs. 20 mg/kg).

As with any clinical trial, the present study had several limitations. Data were obtained only for SDA, and the pharmacokinetic parameters of TMP could not be included. The total dose of TMP was only 4 mg/kg in this study, which was lower than in other studies (Löscher et al., 1990; Baert et al., 2003). Approximately 4–6 h after oral administration of test and reference formulations, the concentration of TMP was below or equal to the LOQ, and TMP could not be detected in the plasma of any birds. It was shown by Löscher et al. (1990) and Baert et al. (2003) that TMP is eliminated rapidly from chicken plasma and TMP exhibited a more extensive tissue distribution than SDA. Even so, the LOD and LOQ values for TMP determined in this study were comparable with those determined by Löscher et al. (1990). Moreover, an accurate bioequivalence study of TMP in plasma requires a more sensitive analytical method as the LOQ of 0.1 mg/L used in this study is clearly not enough for plasma concentration determination.

Additionally, a crossover study is generally used for bioequivalence comparisons because the concentration of drug formulations being compared should neither be known nor influenced by investigators or subjects. However the present study was carried out using a parallel design due to the relatively rapid growth rate and weight gain of broilers. A two-period crossover study with an appropriate inter-period interval would have involved significant body weight gain, and the same birds would not be comparable in the different time periods. Lees and colleagues (2013) investigated pharmacokinetics and bioequivalence of two ivermectin feed formulations in pig using a similar parallel study to that employed in this study with considerable success.

In summary, ANOVA of the AUC, $T_{1/2}$, MRT, C_{max} and T_{max} values calculated in this study showed no significant differences between test or reference preparations. The bioequivalence of I, II and III was confirmed, and these formulations can therefore be used interchangeably in veterinary medical practice.

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