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Bioavailability and Pharmacokinetics of Ampicillin in Chicken Infected with *Eimeria tenella*

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Abstract: Coccidiosis and its following infection with clostridia in chicken are two common diseases in chicken. Ampicillin is a highly recommended therapy in clostridia infections in chicken. However, the effect of coccidiosis on ampicillin pharmacokinetics is not known. In this study, chicken infected with *Eimeria tenella* showed significant changes in ampicillin bioavailability and pharmacokinetics. Compared with noninfected chicken, intravenous injection of ampicillin in the infected chicken showed higher distribution and elimination constants and statistically significant higher AUC and MRT. Furthermore, oral administration in the infected chicken produced higher mean absorption time, delayed t_{max}, lower C_{max}, smaller AUC value and lower bioavailability. Coccidiosis is the major predisposing factor for clostridia infections. Therefore, the use of ampicillin is highly expected following coccidiosis. Based on these results, monitoring and adjustment of ampicillin dosing could be practiced during the presence of coccidiosis.

Key words: Ampicillin, coccidiosis, *Eimeria tenella* pharmacokinetics, bioavailability

INTRODUCTION

Coccidiosis is one of the most important diseases affecting poultry industry. It is one of diseases having high economic impact as it forms about 10% of total broiler mortalities and costs about $3 billion dollars worldwide for the control of the disease (Yadav and Gupta, 2001; Lin *et al*., 2011). Within *Eimeria* species, *Eimeria tenella* (*E. tenella*) is the most pathogenic species by causing severe hemorrhage, marked gain losses and high mortality (Kandeel, 2011; Bacciu *et al*., 2014).

There is a close association between *E. tenella* infection and clostridia enteritis. The presence of coccidiosis is the major cause for growth, proliferation and appearance of clostridia infections in poultry (Cooper and Songer, 2009; Alnassan *et al*., 2014). Mucosal damage by coccidian stages, reductions in intestinal pH and increased transit time, allow proliferation of *Clostridium perfringens* (Al-Sheikhly and Al-Saieg, 1980; Baba *et al*., 1997; Williams *et al*., 2003). The growth and proliferation of *C. perfringens* starts 5 days after coccidiosis and reaches its maximum count at the 6th day of coccidian infections (Baba *et al*., 1988).

There are limited therapeutic choices for treating clostridia infections in chicken with aminopenicillins are the first choice for treatment (Silva *et al*., 2009; Agunos *et al*., 2012). Ampicillin is an aminopenicillin characterized by broad-spectrum antimicrobial activity and high bioavailability (Carceles *et al*., 1996; Escudero *et al*., 2002). Ampicillin showed potent effect on clostridia isolated from chicken (Johansson *et al*., 2004; Gharaibeh *et al*., 2010).

Coccidiosis and clostridia are, therefore, two major concurrent infections in poultry. In such condition, the use of ampicillin is a general trend in the control of coccidiosis. In this perspective, the pharmacokinetics and oral bioavailability of ampicillin in chicken infected with *E. tenella* is not known. In this study, the effect of coccidiosis on the pharmacokinetic properties of ampicillin is investigated. Chicken were experimentally infected with *E. tenella* at the 6th day post-infection (coinciding with high clostridia infection pressure) ampicillin is administered as a single oral or intravenous dose and pharmacokinetic and bioavailability are investigated.

In this study, a trial to mimic the clinical cases was tried. In the field, ampicillin is administered in chicken by oral route. Furthermore, the oral route is preferred over other routes as it will give direct assessment of the effect of GIT damage induced by coccidiosis on ampicillin pharmacokinetics.

MATERIALS AND METHODS

Experimental design: Twenty four one day old chicks were randomly allocated into three groups in a floor pen study. Feed and water are given *ad libitum*. All birds received humanitarian care according to the roles of animal handling instructions. The first group was kept as a control noninfected group and the second and third groups were infected with 40000 oocysts of *E. tenella* at day 14 of age. Six days post infection pharmacokinetics experiment was carried out by administration of 10 mg kg^{-1} ampicillin iv or orally to the second and third groups.

Preparation of infectious *Eimeria* oocycts: Cecal contents from chicken infected with a standard strain of *E. tenella* were collected and examined for the freshly deposited oocysts. Sporulation of oocycts was carried out for several days under continuous aeration from air pump in a solution of potassium dichromate 2.5%. The degree of sporulation was check under microscope. Sporulated oocysts were collected by concentration floatation technique in a concentrated sodium chloride solution. The collected oocycts were washed for several times in a saline solution and kept in a concentration of 40000 oocysts per mL.

Experimental infection: At the age of 14 days, each chick was given 40000 oocycts orally. Chicken were monitored for the clinical sign of coccidiosis at the 3rd and 4th days post infection. Tracing of the oocysts shedding was started at day 5 post infection.

Pharmacokinetic studies: Venous blood samples were collected at 0, 5, 10, 15, 30, 60, 120, 240, 480 min and 12 h after iv or oral administration of the drug. Blood samples were instantly centrifuged and serum was analyzed for ampicillin concentration.

Analytical method: High performance liquid chromatography method was used for analysis of ampicillin concentration as previously described (Apley *et al*., 2007). Shimadzu HPLC system containing Hypersil C18 5 μm particle size reverse phase chromatography column was used to separate ampicillin at a flow rate of 1 mL min^{-1} and the spectrophotometric wavelength was set at 229 nm.

Pharmacokinetic analysis: The time-drug concentration data of ampicillin was subjected to both noncompartmental and compartmental analysis by the aid of PKsolver Excel add-on software (Zhang *et al*., 2010). The parameters calculated included Area Under the Curve (AUC), distribution rate constants (a, b), y intercepts (A, B), half-life ($t_{½}$), volume of distribution (V), Mean Residence Time (MRT), clearance (CL), maximum concentration (C_{max}), time of maximum concentration (t_{max}) and Area Under Moment Curve (AUMC). The area under curve was calculated by a linear trapezoid method. The Mean Absorption Time (MAT) is calculated as:

$$MAT = MRT_{(oral)} - MRT_{(iv)}$$

The bioavailability (F) was calculated as:

$$F = \frac{AUC_{0\text{-}inf} \times dose_{(oral)}}{AUC_{0\text{-}inf} \times dose_{(iv)}}$$

Statistical analysis: Statistical analysis was carried out by using GraphPad Prism 6 package. Pharmacokinetic data were subjected to group multiple t tests to check the levels of significance.

RESULTS

The pharmacokinetic parameters for ampicillin are shown in Table 1 and 2 for iv and oral administrations, respectively. Time-concentration plots are given in Fig. 1 and 2.

After intravenous administration, the distribution half-life was 0.12±0.04 and 0.23±0.13 h for noninfected and infected chicken, respectively. In contrast, the elimination half live of ampicillin was 1.96±0.12 and 3±0.89 h for noninfected and infected chicken, respectively. The Mean Residence Time (MRT) showed significant difference ($p<0.05$) with values of 2.41±0.12 and 3.99±1.05 for noninfected and infected chicken, respectively (Table 1). The calculated AUC was significantly higher in infected chicken (Table 1).

After oral administration, the distribution and elimination half-lives of ampicillin did not show significant changes in both ampicillin-treated and non-treated chicken. The AUC showed significant difference ($p<0.05$) with values of 14.53±1.62 and 13.05±0.84 for noninfected and infected chicken, respectively. Significant differences ($p<0.01$) were observed for AUMC in the infected and noninfected chicken (Table 2). The C_{max} of ampicillin was 2.26 μg mL^{-1} in infected chicken, significantly lower than 3.02 μg mL^{-1} in normal chicken ($p<0.05$). Moreover, the time to reach the maximal plasma concentration was slightly higher in infected than in normal chicken (1.29 and 1.04 h, respectively). The oral bioavailability of ampicillin for normal chicken was 55±1.9. In comparison, infected chicken showed significantly low oral bioavailability ($p<0.05$) of 14±1.7.

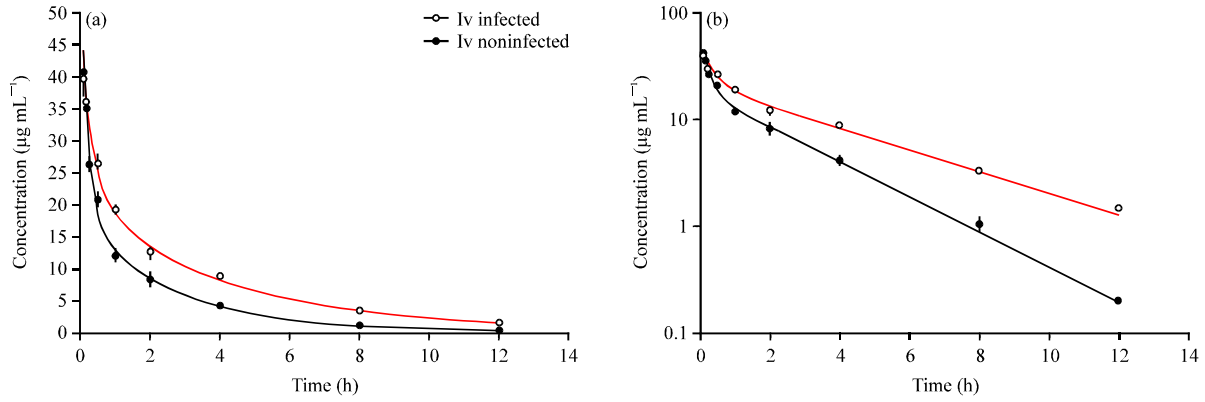

Fig. 1(a-b): Time plasma concentration curve of iv administration of ampicillin (10 mg kg^{-1}) in chicken infected with (a) Cecal coccidiosis and (b) Semi-logarithmic

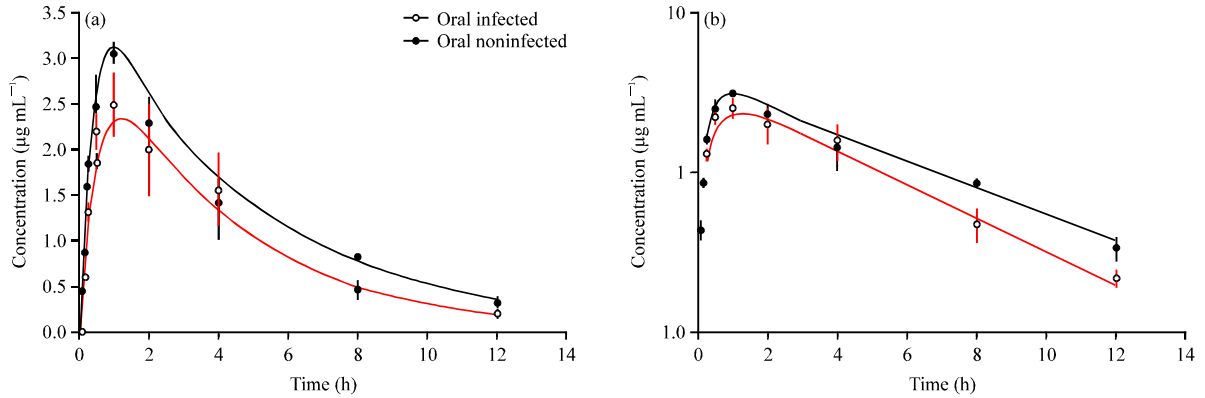

Fig. 2(a-b): Time plasma concentration curve of oral administration of ampicillin (10 mg kg^{-1}) in chicken infected with (a) Cecal coccidiosis and (b) Semi-logarithmic

Table 1: Pharmacokinetic parameters of ampicillin after single intravenous administration in noninfected or chicken infected with *E. tenella*

	Noninfected		Infected	
Parameters	Mean	SD	Mean	SD
A (µg mL^{-1})	34.10	4.38	26.11	2.45
Alpha (1/h)	3.78	0.79	4.38	3.56
B (µg mL^{-1})	16.51	1.68	23.01	6.13
Beta (1/h)	0.35	0.02	0.25	0.07
k10 (1/h)	0.91	0.09	0.48	0.11
k12 (1/h)	1.75	0.47	1.86	1.53
k21 (1/h)	1.47	0.25	2.28	2.00
t1/2Alpha (h)	0.12	0.04	0.23*	0.13
t1/2Beta (h)	1.96	0.12	3.00*	0.19
C0 (µg mL^{-1})	50.61	5.96	49.12	7.22
V (mg kg^{-1})/(µg mL^{-1})	0.20	0.02	0.21	0.03
CL ((mg kg^{-1})/(µg mL^{-1})/h)	0.18	0.01	0.10	0.01**
V2 (mg kg^{-1})/(µg mL^{-1})	0.23	0.01	0.18	0.04
CL2 ((mg kg^{-1})/(µg mL^{-1})/h)	0.34	0.05	0.36	0.24
AUC 0-t (µg mL^{-1}*h)	55.02	1.90	96.71***	5.50
AUC 0-inf (µg mL^{-1}*h)	55.70	1.87	103.22**	10.61
AUMC (µg mL^{-1}*h^2)	134.21	7.58	418.70	152.45
MRT (h)	2.41	0.12	3.99*	1.05

Ampicillin was administered at dose rate of 10 mg kg^{-1} i/v. *p<0.05: Significantly different with noninfected group. **p<0.01: Significantly different with noninfected group. ***p<0.001: Significantly different with noninfected group

Table 2: Pharmacokinetic parameters of ampicillin after single oral administration in noninfected or chicken infected with *E. tenella*

	Noninfected		Infected	
Parameter	Mean	SD	Mean	SD
A (μg mL^{-1})	42.73	14.20	17.92	10.48
Alpha (1/h)	1.27	0.35	0.76	0.40
B (μg mL^{-1})	2.21	1.22	1.84	1.73
Beta (1/h)	0.14	0.05	0.23	0.08
k10 (1/h)	0.36	0.04	0.31	0.08
k12 (1/h)	0.50	0.05	0.15	0.25
k21 (1/h)	0.55	0.42	0.53	0.32
t1/2Alpha (h)	0.57	0.14	1.23	0.90
t1/2Beta (h)	5.01	0.46	3.33	1.35
t1/2ka (μg mL^{-1})	0.50	0.10	0.50	0.15
V/F (mg kg^{-1})/(μg mL^{-1})	1.60	0.15	2.28	0.85
CL/F ((mg kg^{-1})/(μg mL^{-1})/h)	0.57	0.04	0.68	0.09
V2/F (mg kg^{-1})/(μg mL^{-1})	1.98	1.21	0.58	1.01
CL2/F ((mg kg^{-1})/(μg mL^{-1})/h)	0.80	0.13	0.23	0.39
Tmax (μg mL^{-1}*h)	1.04	0.02	1.29	0.30
Cmax (μg mL^{-1}*h)	3.02	0.10	2.26*	0.37
AUC 0-t (μg mL^{-1}*h^2)	14.53	1.62	13.05*	1.52
AUC 0-inf (h)	17.75	1.28	14.93	1.81
AUMC (mg kg^{-1}/(μg mL^{-1}))	127.13	46.81	66.2*	8.2

Ampicillin was administered at dose rate of 10 mg kg^{-1} I/v. *p<0.05: Significantly different with noninfected group. **p<0.01: Significantly different with noninfected group

DISCUSSION

This study was designed to check the effect of coccidiosis on the pharmacokinetics of ampicillin. In this study, the iv and oral routes are used. The iv route will be taken as a standard measurement for ampicillin pharmacokinetics. While, the oral route is advantageous over s/c or i/m route as it considers the effect of coccidiosis on drug absorption and help in determination of dose adjustment policies. Coccidiosis is a common disease in chicken even in applying strict hygienic measures. Furthermore, close association of clostridia infection with coccidiosis gives an importance for knowing the kinetics of anticlostridial drugs during the course of coccidiosis.

The pharmacokinetics of antibiotics in health and disease will help in knowing the treatment strategies and dose optimization during disease conditions.

After oral administration, the time and concentration of maximal ampicillin level in serum showed interesting differences between infected and noninfected chicken. t_{max} was about 1 h in normal chicken which is extended in infected chicken to be 1.29 h. In addition, the maximal ampicillin concentration was lower in infected chicken (Table 2). The Mean Absorption Time (MAT) values were 4.6 h for infected chicken compared to 1 h for noninfected chicken indicating four folds increase in MRT due to infection.

E. tenella infection causes both of local and systemic affections including intestinal inflammation, desquamation of mucosa, liver and renal damage (Kumar *et al*., 2004; Koinarski *et al*., 2005; Zulpo *et al*., 2007; Ogbe *et al*., 2010). The longer elimination half-life, higher t_{max}, lower C_{max}, higher AUC, higher MAT and higher MRT could be caused by the pathological changes associated with coccidiosis.

In the present study, the infection of chicken with coccidiosis noticeably affected the pharmacokinetics of ampicillin in either iv or oral administration. The delayed GIT absorption and lower serum concentration of the administered dose will be accompanied by higher level of the drug in the gut lumen. This may be beneficial in sterilization of gut but the lower serum concentration and lower oral bioavailability may be accompanied by inefficient levels in deeper infected sites. Based on this study, dose adjustments are recommended during oral administration in chicken infected with coccidiosis.

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