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Original Article

Pharmacokinetics of doxycycline hydrochloride in cherry valley duckling during aflatoxicosis

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Abstract: Doxycycline is a time-dependent antimicrobial agent and expected to achieve ideally drug concentration sustained in plasma between dosing intervals. This study was aimed to define its pharmacokinetics in cherry valley ducklings during aflatoxicosis under natural conditions. On 50 μg/kg aflatoxin-contaminated feed, cherry valley ducklings aged from 7 to 21 days were formulated by intragastric administration of a single 20 mg/kg body weight bolus dose of doxycycline hydrochloride. Pharmacokinetic parameters were detected by HPLC. Results showed that main pharmacokinetic parameters in the model group when compared with those of the normal group were: absorption half-life ($T_{1/2\alpha}$) of (3.811±1.607) h vs. (1.431±0.074) h, elimination half-life ($T_{1/2\beta}$) of (29.803±5.750) h vs. (57.142±5.528) h, absorption rate constant (Ka) of (0.251±0.070) h^{-1} vs. (0.602±0.022) h^{-1}, time of maximum plasma concentration (T_{max}) of 3 h vs. 2 h, calculated maximum plasma concentration (C_{max}) of (3.945±0.644) mg/L vs. (1.215±0.058) mg/L, and area under the concentration-time to infinity ($AUC_{0-\infty}$) of (42.520±0.792) mg/L vs. (47.961±0.781) mg/L, respectively. It is indicated that absorption and distribution of doxycycline hydrochloride in cherry valley duckling were slowed due to aflatoxicosis, and liver damage caused by alfatoxin significantly affected doxycycline hydrochloride in enterohepatic cycling, which would accelerate its elimination *in vivo*.

Keywords: Doxycycline hydrochloride, aflatoxicosis, cherry valley duckling, pharmacokinetics, drug

Introduction

Aflatoxins belong to the causative mycotoxins produced by some strains of *Aspergillus flavus* growing on peanut meal. More aflatoxin derivatives, such as aflatoxin B_1 (AfB_1), aflatoxin B_2 (AfB_2), aflatoxin G_1 (AfG_1) and aflatoxin G_2 (AfG_2), have been successively proven to be produced by other Aspergilli on some grains and foodstuffs. The high hepatocarcinogenic toxicity of AfB_1 has been demonstrated in a wide species range and in multiple tissue cultures. Feeds containing detectable amounts of aflatoxins due to improper storage would cause aflatoxicosis in animals.

The duckling is more sensitive to aflatoxins than other young animals, such as chicken [1]. Under long-term diet contaminated by aflatoxin, chronic toxicity could be induced in ducklings, causing loss of growth performance and digestive ability, as well as increased levels of hepatic enzyme indicators and oxidative stress [2]. Generated parenchymal hepatic injuries would ultimately lead to hepatic cirrhosis or hepatoma. Sites of drug metabolism *in vivo* are closely related with blood flow volume of metabolic enzymes in the body, the local organs or tissues. Liver is the major site of metabolism for high blood flow and abundant metabolic active enzymes. Additionally, gastrointestinal is one of the most common metabolism sites [3].

Doxycycline is classified as broad-spectrum semisynthetic tetracycline, which has been widely applied in medical science and veterinarian clinical treatment on account of the high lipid solubility and penetrability, quick absorption for oral administration, strong bioavailability and wide distribution spectrum. Primarily excreted in bile and reabsorbed in the intestine, the distinctive feature of doxycycline is the enterohepatic circulation that is mainly inactivated in integrated form or complexes. When excreted with defecation, the drug would have no significant effect on gastrointestinal flora

Table 1. Composition and proximate analyses of the basal diet

Ingredients	1 to 21 days	22 to 27 days	Nutritive index	1 to 21 days	22 to 27 days
Corn	56	62	Metabolic energy (MJ/kg)	13.18	13.01
Soybean residue	27.3	23.4	Crude protein	22.5	19.8
Corn protein powder	4	4	Calcium	0.98	0.90
Fish protein concentrate	5	3	Phosphorus	0.74	0.70
Vegetable oil	4	4	Lysine	1.17	1.02
Salt	0.3	0.3	Methionine	0.58	0.41
Calcium hydrophosphate	1.5	1.5	Methionine + Cystine	0.90	0.72
Limestone powder	1.2	1.2	Aflatoxin B_1 (μg/kg)	6.0	6.0
Methionine	0.2	0.1			
Multi-dimensional trace elements*	0.5	0.5			

*Multi-dimensional trace elements consisted of 1500 IU Vitamin A, 200 IU Vitamin D3, 10 mg Vitamin E, 0.5 mg Vitamin K, 4 mg Vitamin B, 10 mg d-Pantothenic acid, 2.5 mg Vitamin B6, 25 mg niacin, 800 mg choline chloride, 0.15 mg Biotin, 60 mg Manganese, 40 mg Zinc, 80 mg Iron, 8.0 mg Copper, 0.35 mg Iodine and 0.15 mg Selenium.

and digestive function in animals. But liposoluble doxycycline could be reabsorbed in kidney tubules, which makes it an ideal antimicrobial agent for treatment objects with renal failure or renal insufficiency. Amounts of studies have been conducted on the *in vivo* pharmacokinetics of doxycycline in animals, including pigs, chickens, rabbits, sheep, dogs, cats and cattle etc. [4-9]. In this paper, the pharmacokinetics of doxycycline hydrochloride (20 mg/kg b. w., lavage) in cherry valley ducklings under aflatoxicosis was determined to provide clinical application and guidance of doxycycline hydrochloride with sufficient pharmacokinetic parameters and rational theoretical basis.

Materials and methods

Materials

Standard doxycycline hydrochloride with 85.2% purity was obtained from China Institute of Veterinary Drug Control (IVDC, Beijing). Original powder of doxycycline hydrochloride with 83.6% purity was purchased from Jiupeng Pharmaceuticals (Hubei, China).

Aspergillus flavus

(CICC No. 2219) was obtained from China Center of Industrial Culture Collection (Beijing) and fermented to produce aflatoxin as described [10]. The fermentation of AfB_1 was added into the basal diet to get a concentration of 50 μg/kg and the determination was performed by HPLC.

Animal feeding

120 healthy commercial cherry valley ducklings aged at 1 day were included in the study. According to the feeding request, the ducklings were randomly divided into 2 groups with 60 ducklings per group. Basal diet was formulated in accordance with diet recommended by National Research Council for broiler chicken (**Table 1**). The weak were removed after fed with basal diet for 6 days. The animals in model group were maintained on the basal diet added with 50 μg/kg AfB_1 from 7 to 21 days and fed with basal diet since the 22nd day, companied with vaccinations that included duck rinderpest vaccination, duck virus hepatitis, avian influenza vaccination and any other routine vaccinations. Floor husbandry was adopted in this study and animals had *ad libitum* access to feed and water under 24 h light treatment. The trial period was 27 days. 48 cherry valley ducklings that were accorded to the principle of body weight similarity were finally selected.

Intragastric administration

Serum was collected from jugular vein after ducklings were intragastrically administrated with 20 mg/kg doxycycline hydrochloride for 0 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 60 h and 72 h, respectively. Samples were centrifuged at 4000 r/min for 10 min, and the supernatant plasma was separated and reserved at -20°C.

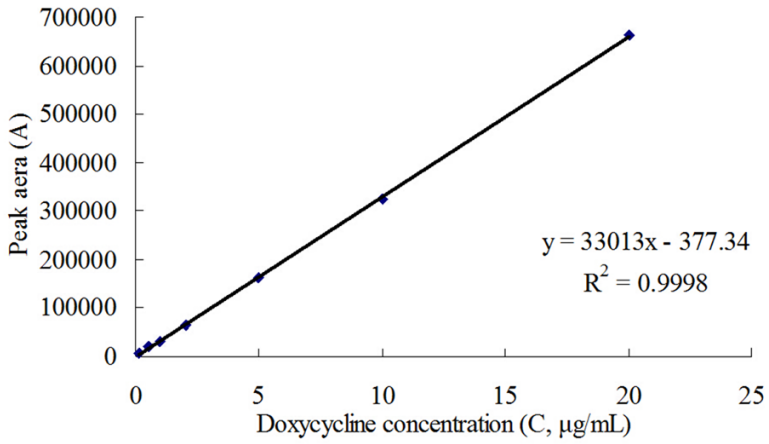

Figure 1. Standard curve of doxycycline hydrochloride. The X axis is the concentration of standard doxycycline and the Y axis is the peak area of doxycycline detected by the HPLC. The fitted equation is established as Y=33013X-377.34 with the R^2 0.9998.

Table 2. Recoveries and precision of doxycycline in plasma

Concentration (μg/mL)	Intra-day precision		Inter-day precision	
	R ± SD/%	CV	R ± SD/%	CV
0.1	92.55±2.33	2.52	95.12±2.13	2.39
1	83.84±2.65	3.16	84.17±2.03	3.82
20	70.50±1.59	2.11	72.76±1.63	2.34

R is short for recovery rate; SD for standard deviation; CV for coefficient of variation.

Extraction and determination

According to the measurements described by Axisa et al [11], 0.5 mL unfrozen plasma under ambient temperature was drawn into EP tube containing 0.3 mL ascorbic acid (0.1 g/mL) and 0.2 mL perchloric acid (18%). Then the deproteinized serum was homogenized in a vortex mixer for 3 min and centrifuged at 12000 r/min for 5 min, and supernatant was filtered through 0.2 μm cylindrical filter membrane to collect filtrate for HPLC detection.

The optimum HPLC conditions to separate doxycycline hydrochloride from solvent and other components in plasma thoroughly were as follows: 0.01 mol/L oxalic acid and acetonitrile (7:3, V:V) consisted the mobile phrase and the flow rate was set as 1 mL/min. Maximum ultraviolet absorption was detected at 350 nm. Column size was 50 μL. Column temperature was 30°C.

Multiple proportion dilutions of the standard stock solution by mobile phrase were 0.1 μg/mL, 0.5 μg/mL, 1 μg/mL, 2 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL and 25 μg/mL, respectively. The linear regression was fitted between peak area (S) and the corresponding doxycycline concentration. Both the standard curve and correlation coefficient were determined by least square method. Each concentration was performed in triplicate.

Peak area of 50 μL treated serum was defined by HPLC to measure doxycycline concentration in plasma from the standard curve. Then plasma doxycycline concentration-time curve was drawn based on the doxycycline concentrations in plasma.

Within the doxycycline hydrochloride concentration in standard curve (0.1 μg/mL to 20 μg/mL), three doxycycline hydrochloride concentrations were acquired and pre-treated to conduct the HPLC operation, which were 0.1 μg/mL, 1 μg/mL and 20 μg/mL, respectively. Each concentration was preformed for five times. Meanwhile, stock solutions at the same concentrations (that were 0.1 μg/mL, 1 μg/mL and 20 μg/mL, respectively) were prepared and analyzed directly. The recovery rate was calculated according to the Equation below:

$$\text{Recovery rate}/\% = \frac{A}{A_s} \times 100\%$$

Where, A is the peak area of doxycycline hydrochloride in plasma sample and A_s is the peak area of doxycycline hydrochloride in the corresponding stock solution. Evaluation of each concentration was repeated for five times. The intra-day precision and inter-day precision were calculated within a week.

Limit of quantitation (LOQ) is defined as the minimal inject quantity of sample when ten times of the signal-to-noise ratio can be detected by the apparatus. And the limit of detection (LOD) is the drug concentration in sample, the minimal inject quantity of which can be detected when three times of the signal-to-noise ratio can be detected.

Data obtained in the paper were processed by DAS 2.0 program software to calculate the

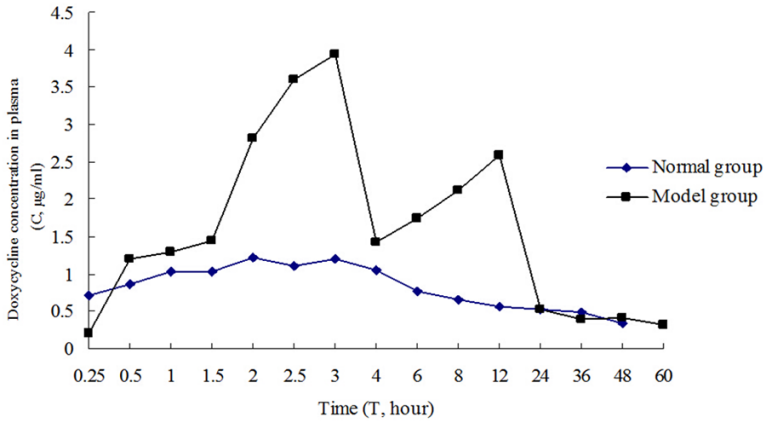

Figure 2. Concentration-time curve of doxycycline hydrochloride. The X axis is the time of oral administration for cherry valley ducklings and Y axis is the doxycycline concentration in plasma. In the figure, stands for the concentration in ducklings from the normal group and stands for those from the model group.

pharmacokinetic parameters. Compartment model was selected based on the minimal AIC and the maximal R^2.

Statistical analysis

The data were presented as mean ± SD. Differences in the drug concentration and the corresponding pharmacokinetic parameters were analyzed by SPSS version 17.0. Statistical comparisons were performed by student's t-test. $P < 0.05$ was considered statistically significant.

Results

The retention time of doxycycline hydrochloride under selected chromatographic conditions was about 7.25 min with good chromatographic peaks. Inside the chromatogram column, the endogenous substance in plasma did not interfere with the determination of doxycycline hydrochloride.

Doxycycline hydrochloride was linear over a range of concentration from 0.1 μg/mL to 20.0 μg/mL by the proposed method (**Figure 1**). The standard equation was demonstrated with the following equation: *Y*=33032*X*-432.0, and the R^2 was 0.999, suggesting that doxycycline hydrochloride in plasma was in good linear relation within the concentration range. The lLimit of detection (LOD) was 0.05 μg/mL, and the limit of quantification (LOQ) was 0.1 μg/mL. As defined in **Table 2**, the measured concentration from the standard equation was compared with the actual concentration to calculate the recovery rate. Average relative recovery rates were 92.55%, 83.84% and 70.50% for doxycycline hydrochloride at concentrations of 0.1 μg/mL, 1.0 μg/mL and 20.0 μg/mL, respectively. Correspondingly, the intra-day relative standard deviations (RSD) were 2.52%, 3.16% and 2.11%, respectively, and those for inter-day were 2.39%, 3.82% and 2.34% respectively. All of them were less than 4% for n=5. These results suggested that the measurement fited the methodology requirements and could be used for determining the drug concentration in plasma.

Figure 2 shows the concentration-time curve of doxycycline hydrochloride in model group and normal group. Models with maximal R^2 and minimal Akaike's information criterion (AIC) were chosen, which conformed to the first-order absorption and two-compartmental model (weight of 1 cc). The fitted models were C=7.925e-0.485t+0.732e-0.012t-8.657e-0.6t and C=1.771e-201t+1.587e-0.024t-3.358e-0.251t, respectively. In model group there was a bimodal phenomenon of doxycycline concentration-time curve in cherry valley ducklings, while there was not in normal group, indicating that distinctive enterohepatic circulation existed during doxycycline hydrochloride metabolism *in vivo* between the two groups. The violate plasma concentration fluctuations in model group might be relevant with hepatic injury caused by aflatoxicosis.

The pharmacokinetic parameters obtained for doxycycline hydrochloride are specified in **Table 3**. Time of maximum plasma concentration (T_{max}) was 3 h for the model group, while it was 2 h for the normal group. The maximum serum concentration (C_{max}) of the model group was (3.945±0.644) mg/L, higher than that of the normal group (1.215±0.058) mg/L. Absorption half-time ($T_{1/2\alpha}$) in the model group was (3.811±1.607) h, twice than the normal group (1.431±0.074) h. These results suggested that the absorption of doxycycline hydrochloride

Table 3. Pharmacokinetics parameters of doxycycline hydrochloride in cherry valley ducklings after oral administration (n=3)

Parameter (h^{-1})	Normal group	Model group	Parameter	Normal group	Model group
α	0.485±0.025	0.201±0.069	K_{21} (h^{-1})	0.166±0.014	0.500±0.016
β	0.012±0.001	0.024±0.005	K_a (h^{-1})	0.60±0.02	0.25±0.05
K_a	0.602±0.022	0.251±0.070	$V_{1/F}$ ($L{\cdot}kg^{-1}$)	10.054±0.631	5.141±1.104
$T_{1/2\alpha}$	1.430±0.074	3.811±1.607	CL/F ($L{\cdot}h^{-1}{\cdot}kg^{-1}$)	0.348±0.120	0.400±0.122
$T_{1/2\beta}$	57.142±5.528	29.803±5.750	T_{max} (h)	2.000±0.000	3.000±0.000
K_{10}	0.035±0.012	0.082±0.039	C_{max} ($mg{\cdot}L^{-1}$)	1.215±0.058	3.945±0.644
K_{12}	0.297±0.002	0.143±0.037	$AUC_{0-\infty}$ ($mg{\cdot}L^{-1}{\cdot}h^{-1}$)	47.961±0.781	42.520±0.792

Footnote: α, Distribution hybrid rate constant; β, Elimination hybrid rate constant; $T_{1/2\alpha}$, Absorption half-life; $T_{1/2\beta}$, Elimination half-life; K_{10}, Elimination rate constant; K_{12} and K_{21}, distributive rate constant; K_a, Absorption rate constant; V1/F, (Apparent volume of unchanged drug distribution in compartment one)/Fractional bioavailability; CL/F, Clearance/Fractional bioavailability; T_{max}, Time of maximum plasma concentration; C_{max}, Calculated maximum plasma concentration; $AUC_{0-\infty}$, Area under the concentration-time to infinity.

was retard in model group. Elimination half-time ($T_{1/2\beta}$) in the model group was (29.803±5.750) h, almost half of the normal group (57.142±5.528) h, indicating that the doxycycline hepatic metabolism was aggravated in model group, as compared with normal group. Both plasma concentration and peak concentration of doxycycline hydrochloride in model group were higher than those in normal group, suggesting that first-pass metabolism was significantly reduced under gastrointestinal and hepatic injuries, leading to more released doxycycline hydrochloride [12]. As shown in **Table 3**, distribution hybrid rate constant (α) and absorption rate constant (K_a) for model group and normal group were (0.485±0.025, 0.201±0.069) h^{-1} and (0.602±0.022, 0.251±0.070) h^{-1}, respectively, which were lower than those for normal group, further illustrating that absorption and distribution of doxycycline in cherry valley duckling was retarded by aflatoxicosis. It corresponds to the T_{max} of 2 h and 3 h for normal group and model group, respectively.

Discussion

In this paper, V1/F was (10.054±0.631) L/kg for ducklings in the normal group and (5.141±1.104) L/kg for those in the model group, suggesting that combination of doxycycline hydrochloride with tissue proteins mainly located within intracellular fluid and intercellular fluid. But in model group, the combination with plasma proteins primarily distributed in the plasma, which was consistent with increased drug excretion and reduced retention time. Moreover, higher doxycycline concentration in plasma was correlated with the weakened enterohepatic circulation that was caused by the dented first-pass metabolism and gastrointestinal injuries [13]. Doxycycline metabolism dependent on its lipid solubility in damaged liver was weakened [14]. As elimination time for model group was much shorter than that for normal group, doxycycline hepatic metabolism in model group was aggravated in spite of the same dosage for oral administration. Area under curve (AUC) is representative of drug bioavailability [15, 16]. Little difference existed between the normal group and the model group, indicating the serum concentration in model group was similar to that in normal group for reduced first-pass effect. Hence aflatoxicosis significantly affect doxycycline metabolism in cherry valley duckling. And it is appropriate that reduction in dosage and increase in administration frequency are recommended for doxycycline in practical application.

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Disclosure of conflict of interest

None.

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