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Pharmacokinetics of Florfenicol and its Metabolite, Florfenicol Amine, in Broiler Chickens

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Abstract : A study on bioavailability and pharmacokinetics of florfenicol was conducted in broilers following a single intravenous (i.v.) and oral (p.o.) doses of 20 mg/kg body weight (b.w.). Florfenicol concentrations in plasma were determined by a high-performance liquid chromatography/mass spectrometry. Plasma concentration–time data after i.v. administration were analyzed by a non-compartmental analysis. Following i.v. injection, the total body clearance was 0.74±0.25 L/kg/h and the volume of distribution at steady-state was 1.16±0.19 L/kg. Florfenicol was rapidly distributed and eliminated following i.v. injection with 1.15±1.06 h of elimination half-life. After oral administration, the calculated C_{max} values (8.18±0.97 μg/mL) were reached at 1.33±0.29 h in broilers. The elimination half-life of florfenicol was 1.31±0.27 h and the absolute bioavailability (F) was 75.46% after oral administration of florfenicol. Florfenicol amine, a major metabolite of florfenicol, was detected in all broilers after i.v. and p.o. administration of florfenicol. The observed C_{max} values of florfenicol amine (3.96±2.60 and 2.22±1.71 μg/mL) were reached at 0.16±0.19 and 1.61±1.02 h after i.v. and p.o. administration of florfenicol, respectively. Florfenicol amine was eliminated with 1.88±0.39 and 2.64±1.39 h of the elimination half-life after i.v. and p.o. administration of florfenicol, respectively.

Key words **:** pharmacokinetics, florfenicol, florfenicol amine, broiler chicken.

Introduction

Florfenicol (d-(threo)-1-(methylsulphonylphenyl)2-dichloro-acetamide-3-fluoro-1-propanol) is a primarily bacteriostatic broad-spectrum antibiotic against many Gram-negative and Gram-positive bacteria (9). Florfenicol was approved world-wide for the control of bacterial respiratory tract infections in cattle and pigs (3,22,26). In food animals, florfenicol has been shown to be effective against bacteria such as *Pasteurella* spp. (9,14,16), *Actinobacillus pleuropneumoniae* (23), *Mycoplasma mycoides* (6), *Staphylococcus aureus* (16), *Salmonella typhimurium* (7) and *Escherichia coli* (9,16).

The pharmacokinetics of florfenicol has been extensively investigated in veal calves (1,24), cows (8,22), horses (17), goats (3,5), pigs (15,26) and broiler chickens (2,21). Florfenicol amine was the major metabolite measured in edible tissues of chicken. The other metabolites, florfenicol oxamic acid, florfenicol alcohol and monochlorofenicol, were also identified[12]. Florfenicol amine is the longest-lived major metabolite in the liver and this was therefore used as the marker residue for withdrawal calculation (19). The aim of this study was to evaluate plasma disposition of florfenicol and its major metabolite, florfenicol amine, after intravenous (i.v.) and oral (p.o.) administrations in broilers.

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Materials and methods

Chemicals

Florfenicol (99.4% assay purity) as analytical standard was purchased from the Sigma (Missouri, USA) and florfenicol amine (99.2% assay purity) was provided by the Zhejiang Hisor Pharm. & Chem. Co., Ltd. (Zhejiang, China). HPLC grade methanol and acetonitrile were purchased from Mallinckrodt Baker (New Jersey, USA). Other analytical grade chemicals were purchased from Sigma (Missouri, USA).

Experimental design

Twenty male broiler chickens (1.59±0.25 kg) were obtained from a commercial farm in Korea. Before the experiment, the animals were acclimatized for 1 week. The birds were monitored daily, and no clinical signs of disease were observed. The room temperature ranged between 20 and 22°C and the relative humidity was maintained at 50-70%. A dark period was given between 0:00 h and 6:00 h. Water and commercial feed were available *ad libitum*. The birds were given a single oral administration directly into the crop using a gavage and a slow i.v. injections into the right brachial vein at the dosage of 20 mg/kg bw of florfenicol. Blood samples (0.5 ml) were drawn from the left brachial vein at 0.08, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 12 h after the intravenous or oral administration. The samples were centrifuged at 1500 g for 10 min to obtain plasma and stored at -70°C until analysis.

Analytical method

Florfenicol and florfenicol amine were extracted from the plasma by the method of van de Riet *et al.* (25). Samples were analyzed on a Hewlett-Packard 1100 series LC/MSD system. Separation was achieved on the Watchers120 ODS-BP C_{18} reverse phase column (5 μm, 4.6×150 mm, Daiso, Japan) and equilibrated with 55% solution A (10 mM ammonium acetate, pH 3.5) and 45% solution B (acetonitrile). The instrument was tuned and optimized for the transmission of the nominal positive ion of florfenicol and florfenicol amine at 359 and 248 m/z. For optimal condition for the analysis of florfenicol and florfenicol amine were employed the pneumatic nebulization with nitrogen (45 p.s.i.) and a counterflow of nitrogen (9 L/min) heated to 350°C for the nebulization and desolvation of the introduced liquid. Mass spectrometery was performed using the positive ion mode and the selected ion monitoring (SIM), detecting m/z 359 (florfenicol) and 248 (florfenicol amine) with peak width of 0.07 and a dwell time of 197 ms. Calibration curves for florfenicol and florfenicol amine were shown linear in the range of 0.01~50 μg/mL with an r^2 value of >0.99. The limit of quantitation for florfenicol and florfenicol amine was 10 ng/mL and the plasma concentrations below this value were not used for the pharmacokinetic analysis. The precision and accuracy for florfenicol were 2.86 to 5.26 and 91.75% to 99.45, respectively. The precision and accuracy for florfenicol amine were 2.58 to 4.26% and 87.75% to 99.45, respectively.

Pharmacokinetic analysis

The data analysis was performed by the non-compartmental analysis using a combined linear trapezoidal rule approach (WinNonlin 4.1, Pharsight, USA). Lambda z is a first-order rate constant associated with the terminal (log linear) segment of the curve. It was estimated by 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 for both i.v. ($AUC_{i.v.0\rightarrow\infty}$) and p.o. ($AUC_{p.o.0\rightarrow\infty}$) studies were calculated by the method of trapezoids. The area under the first moment curve ($AUMC_{0\rightarrow\infty}$) was calculated as the product of time and drug concentration-time. The total body clearance (Cl) was calculated from Cl= $Dose/AUC_{i.v.}$ and the apparent steady-state volume of distribution (V_{ss}) was calculated using $V_{ss}=(Dose_{i.v.})(AUMC)/AUC_{i.v.}^2$. The absolute bioavailability (F) was determined as the ratio (%) of the area under the curve (AUC) after p.o. dosing to that after i.v. dosing. Peak plasma concentrations (C_{max}) of drug and times to reach peak concentration (t_{max}) for the p.o. study were determined from the individual plasma concentration-time curves.

Results

Following i.v. and p.o. administration of a single dose at 20 mg/kg b.w., plasma concentration of florfenicol-time curves were shown (Figs 1 and 2). The pharmacokinetic parameters for florfenicol after i.v. and oral administration at 20 mg/kg b.w. to broilers are shown in Table 1 and 2, respectively.

Following i.v. injection, the Cl was 0.74±0.25 L/kg/h and the Vss was 1.16±0.19 L/kg. Florfenicol was rapidly distributed and eliminated following i.v. injection, with 1.15±1.06 h of $t_{1/2}$.

After oral administration, the observed C_{max} values (8.18±0.97 μg/mL) were reached at 1.33±0.29 h in broilers. The $t_{1/2}$ of florfenicol was 1.31±0.27 h and the calculated F (%) was achieved 75.46% after oral administration of florfenicol.

Florfenicol amine was detected in all broiler chickens after i.v. and p.o. administrations. The Vd and Cl were not calculated for florfenicol amine because it was produced as a metabolite. After i.v. and p.o. administration of florfenicol, the observed C_{max} values of florfenicol amine (3.96±2.60 and 2.22±1.71 μg/mL) were reached at 0.16±0.19 and 1.61±1.02 h,

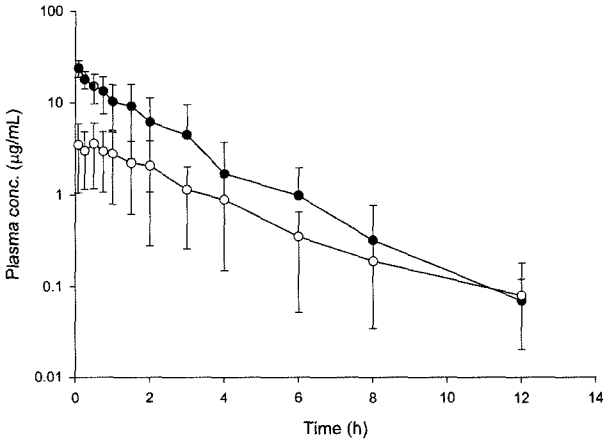

Fig 1. Semilogarithmic plot of florfenicol (●) and florfenicol amine (○) plasma concentration vs. time in broilers after a single intravenous administration at the dose rate of 20 mg/kg (mean± SD, n=10).

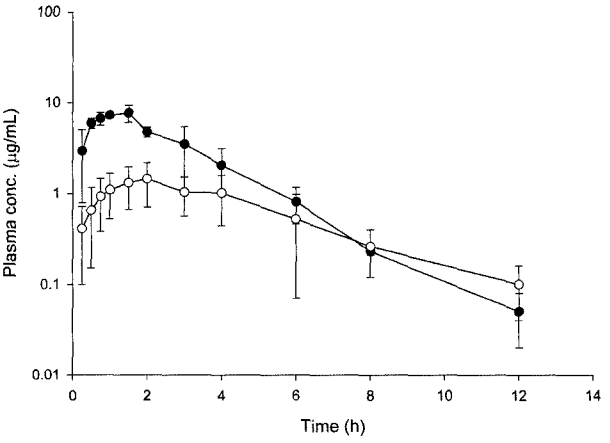

Fig 2. Semilogarithmic plot of florfenicol (●) and florfenicol amine (○) plasma concentration vs. time in broilers after a single oral administration at the dose rate of 20 mg/kg (mean±SD, n=10).

Table 1. Pharmacokincetic parameters determined after intravenous of florfenicol at the dose rate of 20mg/kg to broilers (mean ± SD, n=10)

Parameters (unit)	Florfenicol	Florfenicol amine
	Mean ± SD	Mean±SD
λ_z (1/h)	0.64±0.32	0.38 ± 0.07
$T_{1/2\lambda z}$ (h)	1.15±1.06	1.88±0.39
AUC (μg·h/mL)	30.18±10.41	11.13±9.22
t_{max} (h)	-	0.19±0.09
C_{max} (μg/mL)	-	3.96±2.60
MRT (h)	1.36±0.76	2.55±0.40
V_{ss} (L/Kg)	1.16±0.19	-
Cl (L/kg/h)	0.74±0.25	-

Values are the mean±SD from broilers after i.v. administrations. λ_z, first order rate constant associated with the terminal portion of the curve; $t_{1/2\lambda}$, terminal half-life; AUC, area under curve; t_{max}, time of maximum observed concentration; C_{max}, maximum observed concentration; MRT, mean residence time; V_{ss}, the volume of distribution at steady state; Cl, total body clearance.

Table 2. Pharmacokincetic parameters determined after oral of florfenicol at the dose rate of 20 mg/kg to broilers (mean±SD, n=10)

Parameters (unit)	Florfenicol	Florfenicol amine
	Mean (SD)	Mean (SD)
λ_z (1/h)	0.55±0.12	0.31±0.11
$T_{1/2\bar{e}z}$ (h)	1.31±0.27	2.64±1.39
AUC (μg h/mL)	22.77±2.96	8.24±3.22
t_{max} (h)	1.33±0.29	1.61±1.02
C_{max} (μg/mL)	8.18±0.97	2.22±1.71
MRT (h)	2.47±0.17	4.46±1.95
F (%)	75.46	-

Values are the mean±SD from broilers after p.o. administrations. λ_z, first order rate constant associated with the terminal portion of the curve; $t_{1/2\lambda}$, terminal half-life, AUC, area under curve; t_{max}, time of maximum observed concentration; C_{max} , maximum observed concentration; MRT, mean residence time; F, bioavailability.

respectively. Florfenicol amine was shown slow elimination with 1.88±0.39 and 2.64±1.39 h of $t_{1/2}$ in comparison with its parent drug after i.v. and p.o. administration of florfenicol, respectively.

Discussion

Any side effects associated with the i.v. and p.o. administration of florfenicol in broiles were not shown in this experiment. After i.v. injection of florfenicol, the mean $t_{1/2}$ of the drug in plasma of broilers was 1.51±1.06 h in the present study. It was much shorter than those previously reported in other studies: 2.86-4.11 h in veal calves (1,24), 2.35-2.61 h in goats (3,5), 2.91 h in pigs (15) and 2.80-3.01 h in broiler chicken (2,21).

The V_{ss} is an accurate indication of the diffusion of a drug in body tissues. The pharmacokinetic interpretation of plasma florfenicol concentration data from broilers revealed that florfenicol was widely distributed in well-perfused tissues with the large V_{ss} of 1.16±0.19 L/kg. This value was relatively higher than those reported in veal calves (0.87 L/kg, (24)), lactating cows (0.35 L/kg, (22)), rabbit (0.57 L/kg, (10)). However, the value of V_{ss} in the present study is much lower than reported values in ducks (5.10 L/kg, (11)) and broilers (5.11 L/kg, (2); 4.99 L/kg, (21)).

Bretzlaff *et al.* (8) suggested the small Cl of florfenicol in animals is due to the replacement of -OH in chloramphenicol and thiamphenicol by -F in the florfenicol structure, thereby preventing the conjugation with glucuronic acid and delaying its excretion. In the present study, the total body Cl (0.74± 0.25 L/kg/h) was similar to reported values in duck (0.61 L/kg/h, (11)). It was inconsistent with those reported in broilers (1.56 L/kg/h, (2)). These differences in $t_{1/2}$, Cl and V_{ss} may be related, at least partially, to difference in the metabolism, analytical methods or the metabolic body size of animals involved.

Florfenicol was absorbed rapidly through the gastrointestinal tract with C_{max} of 8.18±0.97 μg/mL at 1.33±0.29 h in broilers. The absolute F of the drug after oral administration of 20 mg/kg b.w. was 75.46%. The oral bioavailabilty was low as compared to the report by Shen *et al.* (20), while greater than that in Affi and Abo el-Sooud (2). This discrepancy may be due the fasting time before dosing. By several mechanisms, food may reduce, delay or enhance the absorption of a drug from the gastro-intestinal tract. For instance, a longer gastric residence time because of the presence of food may lead to better drug dissolution in the stomach and/or in the gastro-intestinal tract, thus resulting in greater F (20). In addition, Varma *et al.* (24) reported that the F of florfenicol was significantly less in non-fasting calves than that in 12-fasting calves before oral administration.

A residue study was conducted by the European Agency for the Evaluation of Medicinal Products (12) on broilers given twice daily 12 hours apart the repeated oral administration of ^{14}C-Florfenicol at 20 mg/kg b.w. for three days. The administered ^{14}C-Florfenicol (93.7 and 98.2%) were excreted within 1 day and 7 days after the last dose, respectively. In excreta, at 7 days, the parent compound represented the major fraction of the radioactivity (42%), and florfenicol amine (25%), florfenicol oxamic acid (5%) and florfenicol alcohol (10%) were also detected. In this study, florfenicol amine was detected in the plasma of broilers after i.v. and p.o. administration. The flofenicol amine was slowly eliminated in comparison with its parent drug with the terminal $t_{1/2}$ of 2.26±0.09 h. These results were in agreement with our previous study for the pharmacokinetics of florfenicol and florfenicol amine in Korean catfish (18).

For bacteriostatic antibiotics including florfenicol, time > MIC (minimum inhibitory concentrations) is, indeed, the most important parameter (4). The MICs of florfenicol for bacteria

isolated from poultry have not yet been determined. Based on MIC data studied on bacteria from fish, swine, calves and cows, 2 μg/ml florfenicol has showed high efficacy against most bacteria (8,13,23). In this study, the time of plasma concentration above 2 μg/mL was approximately 4 h. The pharmacokinetic profile of florfenicol in broiler chickens suggests that it may be therapeutically useful against susceptible microorganisms involved in the most common infections in broilers.

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육계에서의 플로르페니콜 및 그의 대사체인 플로르페니콜 아민의 약물동태학적 연구

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요 약: 본 연구는 플로르페니콜을 체중당 20 mg 용량으로 경구 및 정맥내로 투여한 후 플로르페니콜 및 그 대사체인 플로르페니콜 아민의 생체이용율 및 약물동태학적 분석을 육계에서 실시하였다. 혈청내의 플로르페니콜 및 플로르페니콜 아민의 정량은 액체크로마토그래프/질량분석기를 사용하였으며, 경구 및 정맥내 투여후 혈청 농도-시간 자료는 non-compartmental analysis를 이용하여 분석하였다. 플로르페니콜의 정맥주사 후 청소율 및 소실반감기는 각각 0.74±0.25 L/kg/h와 1.15±1.06 h로 나타났으며, 정상상태 분포용적은 1.16±0.19 L/kg으로 정맥주사후 빠른 체내 분포와 소실을 나타냈다. 플로르페니콜의 경구투여 후 혈중최고농도 (8.18±0.97 μg/mL)는 1.33±0.29 h에 나타났다. 소실반감기는 1.24±0.64 h이었으며, 경구생체이용율은 약 75.46%로 나타났다. 플로르페니콜의 주요 대사체인 플로르페니콜 아민은 정맥 및 경구투여한 모든 육계에서 검출되었다. 플로르페니콜 아민의 혈중최고농도는 정맥 및 경구투여 후 각각 1.88±0.39 μg/mL과 2.64±1.39 μg/mL로 0.16±0.19 h 및 1.61±1.02 h에 관찰되었다. 플로르페니콜 아민은 정맥 및 경구투여후 각각 1.88±0.39 and 2.64±1.39 h로 그 모약인 플로프페니콜보다 다소 느리게 소실되었다.

주요어: 플로르페니콜, 플로르페니콜 아민, 약물동태학, 육계.