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Pharmacomkinetics of Roxithromycin after Intravenous Administration in Broilers

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Abstract : The aim of the present study was to investigate the disposition pharmacokinetics of roxithromycin in broilers. Roxithromycin was administered at a single dose of 20 mg/kg body weight by intravenous (i.v.) routes. Plasma concentrations of roxithromycin were determined by liquid chromatography/mass spectrometry. After a single i.v. dose plasma concentrations were best fitted to a two-compartment open model. The values of the pharmacokinetic parameters after i.v. administration were: elimination half-life = 5.83±1.79 h, mean residence time = 6.33±0.32 h, total body clearance = 0.55±0.15 L/h/kg, and volume of distribution at steady state = 3.47±0.84 L/kg. The pharmacokinetic interpretation of roxithromycin after i.v. administration revealed that the drug was well distributed throughout the body in broilers and slowly eliminated. More studies for the application of roxithromycin against poultry disease are needed to establish a suitable pharmaceutical formulation, propose optimum dosage regimens, investigate clinical efficacy and study the tolerability of repeated doses.

Key words **:** Roxithromycin, pharmacokinetics, broilers.

Introduction

Roxithromycin, chemically designated as (E)-erythromycin-9-[O-[(2-methoxyethoxy)methyl] oxime], is a semisynthetic, orally administered antibacterial macrolide structurally related to erythromycin (3). It has activity against some *Staphylococcus* spp., many *Streptococcus* spp., *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydia trachomatis* as well as many less common organisms (3,11). In comparison with that of erythromycin, its parent compound, the pharmacokinetic profile of roxithromycin is characterized by high plasma, tissue and body fluid concentrations and a long half-life permitting an extended dosage interval (6,11,14). Thus, roxithromycin is an attractive therapeutic alternative for erythromycin in its established indications, especially when the option of lower doses or less frequent administration can be considered. Roxithromycin may be useful in veterinary clinics due to its clinical advantages, but pharmcokinetic profiles of roxithromycin in industrial animals were not fully investigated. Therefore, we investigated the phamrcokinetic profiles of roxithromycin after intravenous (i.v.) administration in broilers.

Materials and Methods

Animals

Twenty healthy broilers, aged 5 weeks, and weighing 1.1~1.3 kg were used in this study. Broilers were housed in stainless steel cages with grate floors. The animals were acclimatized for 1 week before the experiment. Broilers had *ad libitum* access to water and were fed a antibiotics free diet once daily. The broilers had no previous exposure to any antibiotic and no drugs were given to the animals during the acclimation or the study periods. The broilers were determined to be healthy by physical examination.

Experimental Designs

Roxithromycin was dissolved in the combination solution of dimethylsulfoxide (DMSO) and propylene glycol (PG), at the concentration of 200 mg/mL. This formulation used for animal experiments were prepared immediately before administration. Stability of the test compound in the formulation used for intravenous (i.v.) and oral administration to animals was verified for at least 2 h by high performance liquid chromatography/mass spectrometry (LC/MS). Broilers were given single i.v. administrations of roxithromycin 20 mg/kg body weight (b.w.). Venous blood samples were taken from wing vein at 0 (before treatment), 0.25, 0.5, 1, 2, 4, 8, 12, 36, and 48 h. The blood samples were collected in heparinized vacutainers (Becton Dickinson, Rutherford, USA), promptly centrifuged (room temperature, 10 min, 1000 x *g*) and the plasma was stored frozen at -70°C until analysis.

Analytical methods

Roxithromycin were extracted from the plasma as previously reported by our laboratory and assayed by LC/MS (8).

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The limit of quantitation of roxithromycin was 1 ng/mL, the inter-day and intra-day precision (CV, %) was 15% and calibrations were linear ($r > 0.999$) from 0.0001 to 10 μg/mL.

Pharmacokinetic analysis

Monoexponential and biexponential equations were fitted to individual plasma concentration-time data. The plasma concentration vs. time profiles after i.v. administration of roxithromycin was then analyzed by iterative nonlinear least squares regression analysis with equal weighting of the data using a WINNOLIN compartmental model program (Pharsight Corporation, California, USA). The estimation was performed with the use of the weighting model ($1/C^2$ predicted) for the estimation of pharmacokinetic parameters. Compartmental modeling was attempted for the i.v. data, and the best model was chosen using the Akaike's Information Criterion, together with visual inspection of the weighted residual plots (17).

The elimination half-life ($t_{1/2\beta}$) was calculated by $t_{1/2\beta} = 0.693/\beta$. The area under the plasma concentration vs. time curves for both the i.v. ($AUCi.v._{0-\infty}$) study was calculated by the method of trapezoids. The area under the first moment curve ($AUMC_{0-\infty}$) was calculated as the product of time and drug concentration vs. 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).

Antimicrobial effects

Several bacterial strains were obtained from Korea Culture Center of Microorganisms (KCCM, Korea), Culture Collection of Antibiotic Resistant Microbes (CCARM, Korea) and National Veterinary Research and Quarantine Service (NVRQS, Korea). The bacterial strains were grown in trypticase soy broth (Difco, USA) at 28°C in air, resulting in approximately 5×10^9 CFU/ml. The minimum inhibitory concentrations (MICs) were determined by a broth microdilution method in trypticase soy broth (2). Serial two-fold-dilutions of the antibiotics were inoculated with an overnight culture at a final inoculum of 10^6 CFU/mL. The plates were incubated overnight at 37°C. The MIC was defined as the lowest concentration of the antibiotic that prevented visible growth.

Results and Discussion

The LC/MS analysis for the determination of roxithromycin was rapid with a high degree of reproducibility and successfully applied to the pharmacokinetic study of roxithromycin in broilers. The plasma concentrations below limit of quantitation were not used for the pharmacokinetic analysis.

Plasma roxithromycin concentrations after i.v. administration are presented graphically as semi-logarithmic plasma concentrations vs. time plot (Fig. 1). The i.v. data from twenty broilers best fitted a two-compartment open model were suitable for pharmacokinetic analysis of roxithromycin. It shows a rapid rate of drug distribution from the central to the peripheral body compartment. The elimination half-life and the mean residence time (MRT) were 5.83±1.79 h and 6.33±0.32 h, respectively. The V_{ss} and Cl were 3.47±0.84 L/kg and 0.55±0.15 L/h/kg, respectively. A bi-exponential curve of elimination, long half-life and MRT and a large apparent volume of distribution were consistent with a drug which is distributed widely in tissues and then slowly redistributed, achieving moderate but prolonged plasma concentrations (4,13). These findings have been demonstrated by other authors in rats (1,15), dogs (7,18), broilers (8) and humans (5,9,12,16,19).

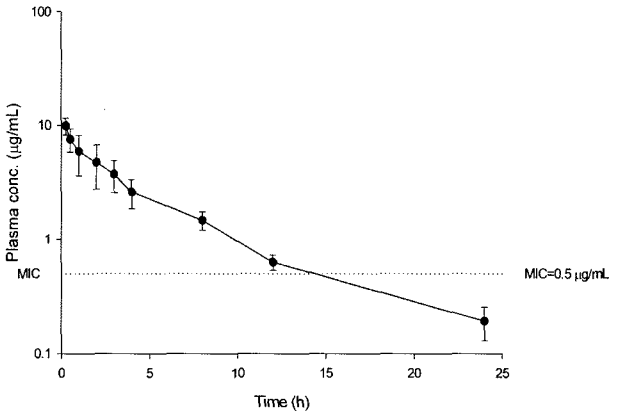

Fig. 1. Plasma concentrations (mean±SD) of roxithromycin in broilers after single intravenous administration at 20 mg/kg body weight.

Research undertaken over the last 15 years has allowed us to define the key pharmacokinetics/pharmacodynamics properties of the main classes of antibiotics that need to be taken into account for optimizing their efficacy (10). For macrolides,

Table 1. Comparative pharmacokinetic parameters (mean±SD) of roxithromycin in broilers after single intravenous administration with a dose rate of 20 mg/kg body weight

Parameters†	Unit	Mean±SD
AUC	μg·h/mL	37.98±10.00
α	L/h	1.52±0.99
β	L/h	0.13±0.03
$t_{1/2\alpha}$	h	0.81±0.81
$t_{1/2\beta}$	h	5.83±1.79
A	μg/L	8.53±1.38
B	μg/L	3.50±1.66
Cl	L/h/kg	0.55±0.15
MRT	h	6.33±0.32
V_{ss}	L/kg	3.47±0.84

†AUC, area under curve; α, absorption rate constant; β, elimination rate constant; $t_{1/2\alpha}$, absorption half-life; $t_{1/2\beta}$, elimination half-life; A, intercept of the absorption phase of bi-exponential curve; B, intercept of the elimination phase of bi-exponential curve; Cl, total body clearance; MRT, mean residence time; V_{ss}, volume of distribution at steady state.

Table 2. MIC of antimicrobial agents against the pathogenic bacterial strains

Bacterial strains[a]	MIC ranges (μg/mL)			
	Roxithromycin	Erythormycin	Tylosin	Enrofloxacin
Escherichia coli CCARM 1250	16	16	32	0.13-0.25
Escherichia coli CCARM 1251	16	16	64	0.06
Escherichia coli KCCM11234	32	32	64	0.03
Staphylococcus aureus KCCM40050	1.25	0.62	8-16	0.13
Staphylococcus aureus CCARM3750	1-4	0.5-4	8-16	0.25
Staphylococcus aureus (3 isolates)	0.5	0.5-1	8	0.13
Staphylococcus aureus (5 isolates)	0.5	0.5-1	8	0.25-0.5
Staphylococcus epidermis KCCM40416	1.25	0.62	8-16	0.13-0.25
Pasteurella multocia TypeD_4	2-8	2-16	8-16	-

[a],*Escherichia coli* CCARM 1250, ECHC isolated from cattle and pigs; *Escherichia coli* CCARM 1251, ECHC isolated from cattle and pigs; *Escherichia coli* KCCM11234, type strain; *Staphylococcus aureus* CCARM 3750, Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from humans; *Staphylococcus aureus* (3 isolates), clinical isolates from broiler; *Staphylococcus aureus* (5 isolates), clinical isolates from subcutaneous abscess in dog; *Staphylococcus epidermis* KCCM40416, obtained from KCCM; *Pasteurella multocia*, obtained from National veterinary Research and Qurantine Service (NVRQS) in Korea.

T > MIC is, indeed, the most important parameter. However, experimental studies show that both T > MIC and AUC_{24h}/MIC influence the clinical efficacy of clarithromycin and azithromycin (13). The T > MIC for about 50% of the dosing interval is suggested sufficient to achieve maximal bactericidal activity in animal study. In the present study, the MICs of roxithromycin for type strains and clinical isolates were given in Table 2 and the antimicrobial activities of roxithromycin were shown similar to erythromycin. Serum concentrations of roxithromycin > 0.5 μg/mL, is the suggested cut-off point separating sensitive from resistant human pathogens (11,14). In the present investigations, the time for which plasma concentration remained above the MIC (> 0.5 μg/mL) and AUC_{24h}/MIC were calculated for predicting the efficacy of roxithromycin in healthy broilers. The T > MIC and AUC_{24h}/MIC after i.v. administration of roxithromycin (20 mg/kg b.w.) were 15.50±2.36 h and 76.80±19.50 h, respectively.

In conclusion, the pharmacokinetic data obtained in this study indicate roxithromycin to be a qualified candidate for the treatment of bacterial infections in broilers. The large volume of distribution and slow elimination of the drug are excellent properties from a therapeutic point of view. However, more studies for the application of roxithromycin in broilers are needed to establish a pharmaceutical formulation, recommended dosages, clinical efficacy and tolerability to repeated doses.

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록시스로마이신의 정맥주사 후 육계에서의 약물동태학적 분석

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요 약: 본 연구는 록시스로마이신의 정맥주사 후 육계에서의 약물동태학적 특성을 조사한것으로, 이때 록시스로마이신은 체중당 20 mg/kg 용량으로 정맥주사하였다. 시간에 따라 채혈하여 혈장을 분리한 후 액체크로마토그래프/질량분석기를 이용하여 혈장내 록시스로마이신의 농도를 측정하였다. 혈장내 록시스로마신 농도-시간 그래프의 분석은 two-compartment open model을 적용하는 것이 가장 적합하였다. 육계에서의 록시스로마이신의 약물동태학적 부변수의 값은 다음과 같았다. 소실 반감기 = 5.83±1.79 h, 평균체류 = 6.33±0.32 h, 청소율 = 0.55±0.15 L/h/kg 및 정상상태 분포용적 = 3.47±0.84 L/kg. 육계에서 정맥주사 후 록시스로마이신은 늦은 소실과 체내 고른 분포의 약물동태학적 특성을 나타내었다. 록시스로마이신의 육계에 적용할 때에는 약물제형, 최적 용량용법, 임상효과 및 반복투여에 대한 내성등의 연구가 추후 요구된다.

주요어: 록시스로마이신, 약물동태학, 육계