Japanese Journal of Veterinary Research 72(3–4): 82–90, 2024

JJVR

REGULAR PAPER Experimental Research

Oral pharmacokinetics and bioavailability of roxithromycin in broiler chickens using HPLC

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Received for publication, August 27, 2024; accepted, October 15, 2024

Abstract

The present study was designed to investigate the oral pharmacokinetics and bioavailability of roxithromycin, a macrolide class antimicrobial drug, in broiler chickens at a single dose of 20 mg/kg body weight. Plasma concentrations of roxithromycin were measured by High Performance Liquid Chromatography (HPLC) method using UV (ultraviolet) detector. The pharmacokinetic (PK) parameters were calculated from plasma concentrations versus time data using 'PK Solver 2.0' software. Following single intravenous administration, the volume of distribution at steady state (V_{ss}) and total body clearance (Cl) were 3.04 L/kg and 0.45 L/hr/kg, respectively. Following oral administration of roxithromycin at the dose rate of 20 mg/kg body weight in broilers, a characteristic short lag phase of 15 minutes in absorption was observed and thereafter mean maximal plasma concentration (C_{max}: 3.60 µg/ml) was achieved at 2 hr. The mean values of elimination half-life ($t_{1/2\beta}$) and mean resident time (MRT) were 8.30 and 9.57 hr, respectively. Calculated oral bioavailability (F) ranged from 51.95 to 62.57% with a mean value of 56.86%. Based on pharmacokinetic-pharmacodynamic (PK-PD) integration efficacy predictor, oral dose at 20 mg/kg body weight of roxithromycin twice a day, is predicted to be effective for treating the susceptible bacterial infections in the broiler chickens with a MIC value ≤ 1.0 µg/ml.

Key Words: Bioavailability, Broiler chickens, Oral, Pharmacokinetics, Roxithromycin

Introduction

The macrolides class of antimicrobials are used for the treatment of diseases that are common in food-producing animals and for mass medication of large groups of birds. In poultry medication, most commonly used macrolides are erythromycin, tylosin and tilmicosin[14]. Tylosin is the only US-FDA (Food and Drug Administration of the United States) approved macrolide to be used in poultry although licensed poultry veterinarians in the United States of America (USA) can use other macrolides as extra-label drug use (ELDU) under the 'Animal Medicinal Drug Use Clarification Act'. Likewise, roxithromycin is not FDA approved for use in poultry but it

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doi: 10.57494/jjvr.72.3–4_82

can be used in an extra-label manner in USA[21]. Roxithromycin is a semi-synthetic, long-acting and orally administered macrolide drug which was derived from erythromycin by altering 14-membered lactone ring to prevent inactivation in the gastric milieu[5]. The antibacterial effect of roxithromycin owes to bacterial protein synthesis inhibition by binding nascent peptide exit tunnel near peptidyl transferase centre at 50 S subunit of bacterial ribosome[9]. Roxithromycin has good lipid solubility with weak ionization property resulting in rapid intracellular accumulation and plateau attainment with high plasma and tissue concentrations[6,18,29]. The tissue : plasma ratios of roxithromycin in various body tissue compartments are generally nearly or larger than one, indicating their high tissue penetration[20]. It has also shown anti-inflammatory activity, independent of its antibacterial activity[22,30]. Roxithromycin is active against Gram-positive and Gram-negative cocci, Gram-positive bacilli and some Gram-negative bacilli. It exhibits good activity against atypical and intracellular pathogens, such as *Mycobacterium avium* complex, *Mycoplasma pneumonia*, *Mycoplasma gallisepticum*, *Helicobacter pylori*, *Chlamydia, Rickettsia*, *Borrelia* and *Legionella* species[5,11]. Based on this antimicrobial spectrum, possible major therapeutic indications of roxithromycin in broiler chicken may include chronic respiratory disease (CRD), infectious coryza, infectious synovitis and necrotic enteritis. Roxithromycin can be more effective than erythromycin and has potential application to be used as an oral medication due to its high stability in the gastric environment, longer elimination half-life and higher plasma levels. Due to ever-increasing antimicrobial resistance problem, there is an imperative need for new effective drugs or to explore the therapeutic implications of existing drugs like roxithromycin in new species. Basic knowledge of oral pharmacokinetic (PK) behaviour of roxithromycin is warranted in broiler chickens which may facilitate exploration of the therapeutic potential of this drug in broiler chickens. In macrolide class, oral PK report of structural analogues of roxithromycin like erythromycin[10], azithromycin[1] and clarithromycin[3] have been reported in broiler chickens but oral PK behaviour of roxithromycin in broiler chickens has been lacking. Only a single report of intravenous pharmacokinetics and a report on residue depletion profile of roxithromycin are available[15,16]. Hence, the present study was undertaken to elucidate the PK of roxithromycin in broiler chickens following single-dose intravenous (IV) and oral administration.

Materials and Methods

Experimental birds

A total of eight healthy male broiler chickens of Vencobb strain aged from 4 to 6 weeks and weighed between 1.4 and 1.8 kg were used for the present experiment. Prior to the experiment, birds were kept under constant observation for 14 days to preclude the existence of any disease or abnormality.

Ethical Approval

The experimentation protocol of the present study was approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary Science & Animal Husbandry, Kamdhenu University, Sardarkrushinagar, India (Approval No. VetColl/IAEC/2016/11/ Protocol-03). All the standard management procedures were followed for taking care of birds as per guidelines of Committee for the Control and Supervision of Experiments on Animals (CCSEA), a statutory committee under Government of India.

Drugs and chemicals

Roxithromycin powder of I.P. (Indian Pharmacopoeia) grade (96.7 % pure) was obtained from Shantam Pharmaceuticals Private Limited, Gandhinagar, Gujarat, India. Water, acetonitrile, trifluoroacetic acid and sodium hydroxide of HPLC grade were procured from S D Fine-Chem Limited, Mumbai, India. For IV dosing, 10 % solution of roxithromycin (100 mg/ml) prepared in 10% dimethyl sulfoxide (final concentration of 10%) and diluted with normal saline was used whereas 2 %

aqueous formulation of roxithromycin (20 mg/ml) was used for oral administration.

Experimental design

Eight healthy broiler chickens were used to study PK of roxithromycin at the dose rate of 20 mg/kg body weight following single dose IV and oral administration in a cross-over design, with the observance of two weeks wash-out time period between two phases of the study. Intravenous injection of the drug (100 mg/ml) was given as IV bolus into wing veins, whereas oral dosing (20 mg/ml) was done using gavage feeding needle. Feed was withheld and birds have fasted 12 hours prior to oral administration of the drugs. About 0.8–1.0 ml blood samples were collected in heparinized test tubes with help of intravenous catheter (22 G, BD Venflon™) placed in the contralateral wing vein at 0 min (pre-administration), 5 (0.083 h), 15 (0.25 h), 30 (0.5 h) minutes. Remaining blood samples were collected at 1, 2, 4, 8, 12, 24, 48 and 72 h. Harvested plasma samples were transferred to cryovials and temporarily stored at -20°C until assayed within a day.

Assay of roxithromycin

Plasma concentrations of roxithromycin were measured using High Performance Liquid Chromatography (HPLC) as per reported method[23]. In brief, HPLC apparatus (Dionex ultimate 3000®, Thermo Fisher, Germany), comprised of ultraviolet (UV) detector, gradient solvent delivery pump and manual injector, was used in the present study. Chromatographic separation was performed by using a reverse-phase C_{18} column (ODS, 25 cm x 4.6 mm ID, 4.5 µm; Purospher® Star RP-18, Merck-Millipore, Mumbai, India) at room temperature (~28°C). The data integration was performed using Chromeleon™ software (version 6.8). The mobile phase consisted of 0.1% trifluoroacetic acid (v/v) buffer and acetonitrile in the ratio of 55 : 45. Erythromycin was used as an internal standard. Isocratic elution mode was employed with a flow rate of 1 ml/min and effluents were monitored at wavelength of 220 nm using UV detector. Lower LOD (limit of detection) and LOQ (limit of quantification) of the method were calculated as 0.131 and 0.398 µg/ml, respectively. The calibration curve was constructed daily using spiked concentrations of 0.20, 0.40, 0.80, 1.60, 3.20, 6.40, and 12.80 mg/ml roxithromycin in plasma and was not accepted unless it had an R^2 value > 0.99.

Liquid-liquid extraction using ice-cold acetonitrile after alkalinisation with 1 M NaOH was performed to extract the drug. Mean extraction recovery of drug from plasma was 80.77 %. For extraction, 400 µL of plasma sample was taken into 2 ml Eppendorf® micro-centrifuge tube, and then 40 µL 1 M NaOH was added to it and vortexed for about 10 seconds at room temperature. After this alkalinization, 1200 µL ice-cold acetonitrile was added in same tube and vortex mixed for 3 minutes. Then the mixture was centrifuged at 2856 g for 10 minutes at 4°C. Resultant upper organic phase was transferred into evaporation vials and completely dried under nitrogen gas flow using a sample evaporator (10 psi pressure, 50°C, 30 minutes). Exactly, 20 µL of internal standard (200 mg/ml erythromycin) was mixed with 80 µL diluent (50% acetonitrile and 50 % HPLC grade water) and same was used for reconstitution of each evaporated sample. Thus, dried residues were reconstituted with 100 µL diluent. The prepared sample was finally centrifuged (2856 g, 5 minutes, 4°C) and 20 µL of the clear supernatant was manually injected into the HPLC machine.

Pharmacokinetic analysis

The plasma concentration-time curves of individual broiler chicken were subjected to non-compartmental analysis (NCA) and various pharmacokinetic parameters were computed with the PK Solver 2.0 software[31]. NCA is the preferred methodology over compartmental analysis to determine the degree of exposure following administration of a drug and associated pharmacokinetic parameters of drugs as it requires fewer assumptions than model-based approaches[8]. Oral bioavailability (F) for individual animals was calculated as follow[4]:

$$F\ (\%) = \frac{AUC_{PO}}{AUC_{IV}} \times \frac{IV\ Dose}{PO\ Dose} \times 100$$

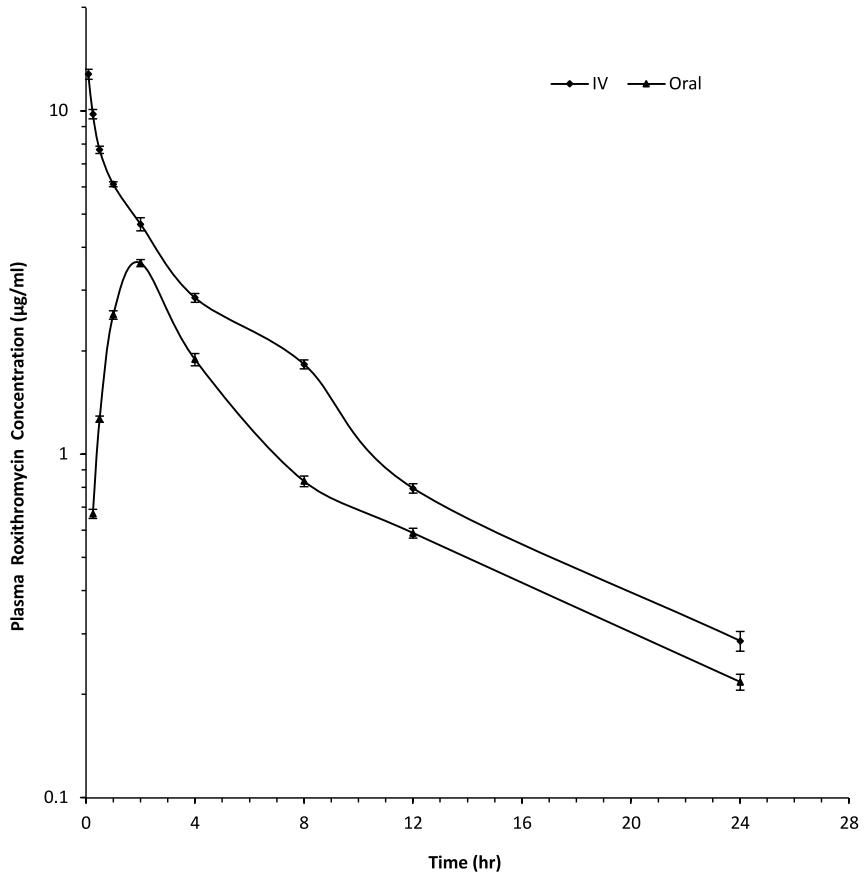

Fig. 1 The Semi logarithmic plot of plasma roxithromycin concentration (mean ± SEM) versus time following IV and oral administration at the dose rate of 20 mg/kg body weight in broiler chickens (n=8).

Statistical analysis

All the pharmacokinetic values were expressed as the mean ± SEM (standard error of the mean) for data of all eight individual broiler chickens.

Results

The graphical representation of plasma roxithromycin concentrations versus time as semi-logarithmic plot after single dose IV and oral administration at the dose rate of 20 mg/kg body weight in broiler chickens is depicted in Figure 1. Following IV administration, mean roxithromycin concentration in plasma at first collection time point i.e. 0.083 hr (5 min) was found to be 12.77 µg/ml which was observed to be 0.29 µg/ml at 24 hr. Following oral administration, mean plasma drug concentration reached to the peak level (C_{max}: 3.60 µg/ml) at 2 hr. The lowest detectable mean concentration was 0.22 µg/ml found at 24 hr. Roxithromycin was not detectable in plasma beyond 24 hr following either IV or oral administration. The PK parameters of roxithromycin derived for broiler chickens following IV and oral administration are presented in Table 1. The values of apparent volume of distribution (V_{area}) and total body clearance (Cl) following oral drug administration were specified relative to bioavailability (F) by PK Solver 2.0 software and were reported as Varea/F and Cl/F values, respectively.

Discussion

Roxithromycin is an oxime derivative of erythromycin and used as an over-the-counter drug in poultry industry. In a study conducted on pattern and determinant of antibiotics use on

Table 1. Pharmacokinetic parameters of roxithromycin (20 mg/kg body weight) following its single-dose IV and oral administrations in broiler chickens (n=8).

Pharmacokinetic parameters	Unit	Values (Mean ± SEM)	
		IV administration	Oral administration
ß	Per h	0.13 ± 0.00	0.08 ± 0.00
C_{max}	µg/ml	-	3.60 ± 0.09
T_{max}	h	-	2.00 ± 0.00
$t_{1/2ß}$	h	5.55 ± 0.19	8.30 ± 0.25
$AUC_{0-\infty}$	µg·h/ml	44.98 ± 0.99	25.59 ± 0.85
AUMC	µg·h^2/ml	305.22 ± 8.53	245.91 ± 12.98
MRT	h	6.80 ± 0.21	9.57 ± 0.24
V_{ss}	L/kg	3.04 ± 0.14	-
V_{area}	L/kg	3.58 ± 0.18	-
V_{area}/F	L/kg	-	9.39 ± 0.24
Cl	L/h/kg	0.45 ± 0.01	-
Cl/F	L/h/kg	-	0.79 ± 0.03
F	%	-	56.86 ± 1.21

SEM: Standard error of the mean, β: Elimination rate constant, C_{max}: Observed peak plasma concentration, T_{max}: Time at which C_{max} was observed, $t_{1/2ß}$: Elimination half-life, $AUC_{0-\infty}$: Area under curve, AUMC: Area under first moment of the plasma drug concentration, MRT: Mean Resident Time, V_{area}: Apparent volume of distribution, V_{ss}: Volume of distribution at steady state, Cl: Total body clearance, F: Bioavailability

101 broiler farms in Thailand, roxithromycin was reported to be most commonly used macrolide antibiotics and it was among top five antibacterial drugs used[19]. It has the potential to be used to treat avian mycoplasmosis or CRD in broiler birds. Like other macrolides, this drug is likely to be used at high doses in poultry to treat atypical pathogens like *Mycoplasma* spp. against which roxithromycin has high MIC value[11]. Therefore, the present PK study was undertaken at the dose rate of 20 mg/kg body weight. Single dose PK study of other macrolides like erythromycin and azithromycin was too reported previously in broiler chickens at high doses of 30 and 20 mg/kg body weight, respectively[1,10]. In the present study, following single dose IV administration at 20 mg/kg body weight, a high initial plasma concentration of roxithromycin was observed as 7.70 µg/ml at 0.5 hr, indicating high plasma concentration/ MIC ratio in the initial phase of disposition kinetics. At such high concentration, a drug of macrolide class, like roxithromycin, is expected to have bacteriocidal rather than bacteriostatic effect[5]. The elimination rate constant (ß) varied from 0.11 to 0.14 per hr with a mean value of 0.13 per hr and its corresponding mean half-life was 5.55 hr with a range of 4.87 to 6.20 hr. Almost similar mean values of elimination half-life for roxithromycin (5.83 hr) and clarithromycin (4.58 hr) were reported in broiler chickens following IV injection[3,15], but almost three times longer elimination half-life value ($t_{1/2ß}$ = 16.27 hr) was reported in Beagle dogs[17]. The species variation for half-life is clearly evident and this may be due to variation in metabolism and excretion patterns of the drug among dogs and broiler chickens.

The mean values of apparent volume of distribution (V_{area}) and volume of distribution at steady state (V_{ss}) were 3.58 and 3.04 L/kg, respectively. High volumes of distribution were endorsing the wide distribution and powerful penetration properties of roxithromycin. Still higher V_{ss} values were reported for other semi-synthetic macrolides like azithromycin (47.75 L/kg) and gamithromycin (27.08 L/kg) in broiler chickens administered by IV route at the dose rate of 20 and 6 mg/kg body weight, respectively[1,28]. The mean value for total body clearance (Cl) in the present study was 0.45 L/hr/kg (7.5 ml/min/kg). The clearance or roxithromycin in broilers in another study was 0.55 L/hr/kg and in dogs, it was 0.21 L/hr/kg[15,17]. Rapid rate of drug clearance from the body of broiler chiickens seems to be the major reason for the shorter elimination half-life of roxithromycin. Thus, there was a marked interspecies difference in the pharmacokinetics of roxithromycin between dogs and broiler chickens.

In the present study, a short lag phase of

15 minutes in oral absorption was observed for roxithromycin before it enters into the blood circulation. It was evident by the absence of detectable concentration of the drug in the samples collected at 5 min (0.083 h) and presence of a low concentration of roxithromycin (0.67 μg/ml) at 15 min post oral administration. Similarly, a lag phase of 30 minutes in oral absorption was also observed for clarithromycin in broiler chickens[3]. Delay in drug absorption may be attributed to the incomplete wetting of drug particles owing to the hydrophobic nature of the drug[12] or due to altered physiological processes like delayed gastric emptying. Short lag phase noticed in the present study may be due to the large size and hydrophobicity of the roxithromycin molecules. Following oral administration, plasma roxithromycin concentration increased gradually after 30 minutes and reached to the peak level (3.60 μg/ml) at 2 hr and then rapidly declined to about half concentration (1.89 μg/ml) in next 2 hr, which indicates rapid distribution of the drug occurred once it attained adequate concentration in circulation. In terminal phases, a gradual decline in drug concentration was observed and at 24 hr, a low mean concentration of drug (0.22 μg/ml) was observed. No roxithromycin concentrations were detectable in plasma samples collected beyond 24 hr and similar finding was also reported for erythromycin in broiler chickens where drug was not detected after 24 hr[10].

The mean value of elimination rate constant was found to be 0.08 per hour and corresponding values of elimination half-life ranged from 7.21 to 9.23 hr with a mean value of 8.30 hr. There is wide variation in the reports for the elimination half-life of different macrolides in broiler chickens viz. 4.1 hr for erythromycin[10], 2.11 hr for clarithromycin[3] and 31.50 hr for azithromycin[1] in comparison to 8.30 hr for roxithromycin in the present study. Thus, in broiler chickens, roxithromycin showed longer elimination half-life than erythromycin and clarithromycin but shorter half-life than azithromycin. The roxithromycin drug molecules stayed for a substantial average time of period in the body of broiler chickens following oral dosing as reflected by high mean resident time (MRT) values observed in the present study which ranged from 8.60 to 10.63 hr with a mean value of 9.57 hr. The average apparent volume of distribution relative to bioavailability (V_{area}/F) following oral administration of roxithromycin was found to be 9.39 L/kg, more than the V_{area} value of roxithromycin (3.58 L/kg) obtained following IV route. Mean value for total body clearance relative to bioavailability (Cl/F) after oral dosing was 0.79 L/hr/kg (*i.e.* 13.1 ml/min/kg); higher clearance rate was reported for azithromycin (0.91 L/hr/kg) in broiler chickens[1]. Moderate to high clearance rate, as reported in the present study, is a favourable PK attribute for the drugs to be used in food-producing animals or birds, as clearance indicates how efficiently and rapidly a drug is eliminated from the body. In a residue study, the optimal withdrawal time of roxithromycin was suggested to be 7 days for edible tissues of broiler following treatment of roxithromycin (at a dose rate of 15 mg/L given for 7 days) mixed with drinking water[16] which concur to the use of this drug in broiler chickens.

The mean ± SEM value of oral bioavailability of roxithromycin in the present study was 56.86 ± 1.21 % and individual bioavailability values dwell in the range of 51.95 to 62.57 %. In broiler chickens, the oral bioavailability of tylosin (another macrolide) was reported low (30–34 %)[13] to very high (90.29 %)[25]. Higher oral bioavailability figures were reported as 83.52 % for azithromycin[1] and 66.54 % for clarithromycin[3] in broiler chickens. However, the moderate oral bioavailability of roxithromycin found in the present experiment is good enough and encouraging for oral dosing of roxithromycin in broiler chickens.

For macrolides (time-dependent antimicrobials) like roxithromycin, the best PK-PD integration indices to predict efficacy is 'time above the MIC (T>MIC)' whose desired value should be 50–80% (at least 40–50 %) of the dosage interval[2,7,26]. For oral dosage regimen determination of roxithromycin in broiler chickens from the present study, the time for which the plasma drug concentrations remain above or equal to minimal inhibitory concentration (MIC) value (T>MIC) was calculated using the following formula[27]:

$$\%\, T > MIC = \left(\ln \frac{\mathrm{D}}{V_{d(area)} \times \mathrm{MIC}} \right) \times \frac{t_{1/2\beta}}{\ln(2)} \times \frac{100}{\mathrm{DI}}$$

where, T > MIC is the time interval (in %) during which the plasma concentration is above or equal to the MIC values; ln is natural logarithm; D is the proposed dose; V_{area} is the apparent volume of distribution; $t_{1/2\beta}$ is the elimination half-life, and DI is dose interval.

A MIC value of 0.5 µg/ml covers most of the pathogenic bacteria for roxithromycin[6,29], however, MIC cut off value equal to 1.00 µg/ml was considered for calculation as it also covers *Mycoplasma gallisepticum* in poultry[11]. Using the above formulae, it was derived that roxithromycin given at the dose rate of 20 mg/kg body weight in broiler chickens, if repeated every 12 hours, gives the satisfactory T>MIC value of 75.45 %, for the susceptible bacteria having MIC up to 1.00 µg/ml. The safety of roxithromycin at such dosage regimen in broiler birds has been reported recently[24].

To summarize, based on favourable pharmacokinetic data obtained in the present study, it seems that roxithromycin may prove to be useful as an antimicrobial drug to treat the diseases caused by susceptible pathogens in broiler chickens. Based on the calculated value of T>MIC (%), it would be prudent to use roxithromycin at the oral dose of 20 mg/kg body weight of broiler chickens, to be repeated every 12 hr, for combating common susceptible pathogens including *Mycoplasma gallisepticum* in broiler chickens. All the broiler chickens administered with single dose roxithromycin either via IV or oral route were showing normal demeanour during the whole experimentation period without exhibiting any signs or symptoms of adverse drug reactions or side effects. The appetite, water and feed intake, defecation, gait and alertness were found to be normal on clinical observations.

Acknowledgements

The authors are thankful to the Department of Livestock Production & Management, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat, for providing the experimental broiler chickens for the present study.

Conflict of Interest

The authors declare that there is no conflict of interests in publishing this work.

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