Animal Health Research Journal Vol. 7, No. 1, March 2019 pp. 125-136

ISSN: 2356-7767

Studies on the effect of co-administration of fluconazole on distribution, elimination and residues of clarithromycin in rabbit tissues

Hesham, S. Taha and Riham, M. Raafat

Biochemistry, Toxicology and Feed Deficiency Dept. (Pharmacology Unit), Animal Health Research Institute, Giza, Egypt

Received in 6/1/2019
Accepted in 18/2/2019

Abstract

The pharmacokinetic and residual studies of clarithromycin (10 mg kg^{-1}b.wt.) were studied following intramuscular administration of clarithromycin alone and co-administered with fluconazole (10 mg kg^{-1}, intramuscularly). The serum and tissue concentrations of clarithromycin were determined by microbiological assay technique using *Bacillus subtilis* as test organism. Following IV administration of clarithromycin, the disposition curve was best described by two-compartment open model. The serum concentration was significantly higher in clarithromycin-fluconazole group than clarithromycin group following IM and IV routes. Clarithromycin was eliminated slowely ($t_{1/2\beta}$, 3.38 ± 0.31h) with mean residence time was 3.77±0.19h after IV route. After IM administration, the maximum serum concentration (C_{max}) and area under the curve (AUC_{0-t}) are significantly higher in clarithromycin- fluconazole group. Clarithromycin achieved its maximum plasma concentrations (C_{max}) of 1.23 ± 0.01µg/ml at maximum time (T_{max}) of 1.23 ±0.01h in clarithromycin group while C_{max} was 1.42 ± 0.02 µg/ml at T_{max} of 1.24±0.01 h in clarithromycin- fluconazole group. The C_{max}/ MIC ratio were 12.3 and 14.2 in clarithromycin and clarithromycin- fluconazole groups respectively, which indicates a potential clinical efficacy against bacterial infection with MIC_{90} > 0.1 µg/ml. **In conclusion,** fluconazole can be used effectively along with clarithromycin to treat the drug sensitive microbial infections associated with fungal infection. Clarithromycin at a dose of 10 mg kg^{-1} b.wt. administered intramuscularly at 12 hrs intervals was recommended in rabbits. Based on the tissue concentration in edible organs a 4 days withdrawal time is suggested.

***Key words**: Clarithromycin, Fluconazole, Pharmacokinetics, Tissue residues.*

Introduction

Although rabbit meat production represents a very small percentage of the world meat market, this percentage has been growing continuously during the last years. Rabbit is considered a minor food species, and therefore no drugs are specifically registered for this animal. This situation encourages rabbit farmers to make off-label use of antibacterial drugs authorized for food-producing animal species other than rabbits or using human medicines in treating specific diseases. In addition, rabbits used as a human and animal model for experimental research. Clarithromycin and Fluconazole are human medicines that can be used to treat bacterial and fungal diseases, respectively in rabbits.

Fluconazole is a new antifungal agent, a triazole derivative that has a broad spectrum of activity against most candida species and other fungal organisms (**Garcia-Cuesta *et al.,* 2014** and **Goins *et al.,* 2002**). It has an excellent tissue penetration, especially in the ocular tissue and central nervous system, when administered orally **(Felton *et al.,* 2014** and **Haynes *et al.,* 2002).**

Fluconazole is effective for treatment of canine nasal aspergillosis **(Belda *et al.,* 2018)**, canine central nervous system cryptococcosi **(O'Toole *et al.,* 2003)**, canine blastomycosis **(Mazepa *et al.,* 2001)**, cryptococcosis in cats (**Pennisi *et al.,* 2013**) and candida endophthalmitis in rabbits (**Riddell *et al.,* 2011**).

Clarithromycin is a macrolide antibiotic and an acid-stable analogue of erythromycin consisting of a 14-membered lactone ring with substitution of a methoxy group from the C-6 hydroxyl group of erythromycin **(Cyphert *et al.,* 2017).** Clarithromycin has bactericidal activity against both typical and atypical respiratory pathogens **(Blasi, 2004).**

The pharmacokinetics of clarithromycin has been extensively investigated in broiler chickens **(Hanady *et al*., 2016**) and in foals (**Jacks *et al.,* 2002**). The combination of Clarithromycin and rifampin is synergistic both in vitro and in vivo, reduces the likelihood of *Rhodococcus equi* resistance to either drug **(Steeve, 2010).**

In some cases it may be forced to use antibiotic and antifungal agents in mixed infections .In immunocompromised patients, fungal infections are often accompanied by bacterial infections. In this respect, the understanding of possible synergistic interactions between antibacterial and antifungal agents against pathogenic fungi is of great importance **(Liu *et al.,* 2014).**

Mixed fungal-bacterial lung infection that cause pneumonia in calves that did not respond to the treatment with florfenicol alone **(Aslan *et al.,* 2002**). In contrast, though many cystic fibrosis patients have airway infections characterized by the presence of both bacteria and fungi **(Laurie and Deborah, 2010).**

Because of limited data on the effect of antifungal agent on the pharmacokinetic of antibiotic and the proper use of clarithromycin in rabbits, this study was designed to determine the pharmacokinetics and the residual studies of clarithromycin to estimate an appropriate dosage regimen and the proposed withdrawal period. Moreover, to investigate the effect of co-administration of fluconazole on distribution, elimination and residues of clarithromycin in rabbit tissues.

Materials and Methods

Drugs

1- **Clarithromycin** (Klacid, Manufacturer is Abbot/France), supplied as a lyophilized form in a 10-mL vial equivalent to 500 mg of clarithromycin. Reconstitution according to label directions, resulting in approximately 50 mg/ ml.

2- **Fluconazole** (Diflucan, Manufacturer is Fareva Amboise / France under authority of Pfizer- france), 50 ml injectable solution, each 1 ml contain 2 mg fluconazole .

Experimental animals

Fifty healthy rabbits, weighing 2.5-3 kg were used. Rabbits were housed in cages, fed on balanced drug free-ration for two weeks before the experiment and supplied with water *ad-libitum*.

Experimental design (for pharmacokinetic study)

Rabbits were divided into four groups of Five each:

Group I: was administered with a single intravenous dose of clarithromycin at 10 mg kg^{-1} b.wt. (into the ear vein).

Group II: was injected with a single intramuscular dose of clarithromycin at 10 mg kg^{-1} b.wt. (into quadriceps femoris muscle).

Group III: was administered with a single intravenous dose of clarithromycin at 10 mg kg^{-1} b.wt. co-administered with a single intramuscular dose of fluconazole at 10 mg kg^{-1} b.wt.

Group IV: was administered with a single intramuscular dose of clarithromycin at 10 mg kg^{-1} b.wt. co-administered with a single intramuscular dose of fluconazole at 10 mg kg^{-1} b.wt.

Tissue distribution (repeated IM administration for drug residual study)

Thirty rabbits were divided into two groups of

fifteen each. Group V was injected intramuscularly with clarithromycin (10 mg kg^{-1} b.wt) once daily for five successive days. Group VI was injected clarithromycin (10 mg kg^{-1} b.wt.) intramuscularly with fluconazole (10 mg kg^{-1} bwt, intramuscularly) once daily for five successive days.

Five rabbits from each groups were slaughtered at 1, 3, 5 days after the last dose. Tissue samples from liver, thigh muscle, lung, kidney, heart, spleen were taken from each slaughtered rabbit. One gram of tissue was grinded with 5 ml of distilled water and centrifuged at 1500*g* for 30 min and stored at -20^0 C until used **(San Martín *et al.,* 2007).**

Blood samples

About one milliliter of blood was taken from the right ear vein at 5, 10, 15, 30 min. and 1, 2, 4, 8 and 12 h in all groups post injection for pharmacokinetic study. Also, following repeated IM administration, blood samples were collected at 1, 3, 5 days after the last dose. All blood samples were centrifuged at 3500 rpm for 10 minutes and serum was harvested and stored at -20^0 C until assayed.

Assay of clarithromycin

Serum and tissue concentrations of clarithromycin were measured by microbiological assay technique using *Bacillus subtilis* ATCC 6633 as test organism **(Abo-El-Sooud *et al.,* 2012)**.

Standard curve of clarithromycin

For establishment of standard curve of clarithromycin, a stock solution of 100µg/ml of clarithromycin in distilled water was prepared. Standard concentrations were obtained by further dilution in distilled water or in drug free chicken serum to obtain concentrations of 0.312, 0.625, 1.25, 2.5, 5 and 10µg/ml. Clarithromycin concentrations in the test samples (serum or tissues) were calculated from the standard curve (**EL. Sayed *et al.,* 2018)**.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameter calculated by PK Solver: An add-in program for Microsoft Excel, version 2 **(Zhang *et al.,* 2010).** The data generated were subjected to statistical analysis employing the Student′s t-test with $P<0.05$ as the level of significance **(Snedecor and Cochran, 2014) .**

Results and Discussion

The pharmacokinetic drug interaction between agents used as part of a multidrug regimen which are important because the interaction may have influence on drug efficacy. Multidrug regimens are often administered to animals for the duration of their lives.

The mean (±SD) pharmacokinetic parameters based on compartmental pharmacokinetic analysis method are presented in (Tables 1 and 2). Clarithromycin serum concentration versus time data after I.V. administration were best fitted to a two-compartment open model (Figure 1). The results revealed that plasma clarithromycin concentration versus time decreased in a bi-exponential manner, demonstrating the presence of distribution and elimination phases. This finding is in agreement with that of clarithromycin in other species including foals **(Womble *et al.,* 2006)**, rats **(lee *et al.,* 2004)** and in broiler chickens **(Hanady *et al.,* 2016).**

The serum concentrations of clarithromycin with fluconazole group was significantly greater than those reported in clarithromycin alone at the times of sample collection after I.V. and I.M. injection (Figure 1 and 2). This results are consistent with that reported by **Ville-Veikko *et al.* (2006)**, where they found that the antifungals, voriconazole and fluconazole significantly increase the plasma concentrations of S-(+)-ibuprofen but have only a weak effect on the pharmacokinetics of R-(−)-ibuprofen**.**

After IV administration, The absorption rate constant (K_{ab}) and the value of distribution rate constant (α), was significantly higher in Group I, however, clarithromycin was rapidly distributed in both groups (Group I and III) with short $t_{½\alpha}$ (0.30±0.02 h and 0.34±0.02 h respectively). The rapid distribution of clarithromycin was further supported by high value of K_{12} (1.14±0.07 $h^{-1}$and 0.89±0.12 h^{-1}) in both groups (I and III) respectively, significantly

higher in Group I.

The first order transfer rate constant for drug distribution from central to peripheral compartment (K_{12}) (1.14 ± 0.07 h^{-1}, 0.89 ± 0.12 h^{-1}) was higher than k_{21} (0.54 ± 0.06 h^{-1} and 0.55 ± 0.08 h^{-1}) in groups I and III, indicate that clarithromycin was transferred from central to peripheral compartment at a faster rate than its passage from peripheral compartment the drug was rapidly distributed with a short T 1/2α (0.30+0.02h) in groups I and III to central compartment. The drug was rapidly distributed with a short $t_{½\alpha}$ (0.30 ± 0.02 h, 0.34 ± 0.02 h) in groups I and III. This value was in consistent with that reported by **(Hanady *et al.,* 2016)** as they found that $t_{½\alpha}$ for clarithromycin in broiler chickens was 0.38 h.

The total clearance of a drug from the body is expressed in terms of the volume of blood cleared of the drug by the various elimination processes (biotransformation and excretion) per unit time and body weight. Therefore, it is a measure of the ability of the organs of elimination to remove drug from the plasma . In the present study, the total body clearance (Cl) of clarithromycin was 0.74 ± 0.01 (mg)/(µg/ml)/h was significantly faster to those reported in clarithromycin–fluconazole group was 0.66 ± 0.02 (mg)/(µg/ml)/h. The value may be close to that reported in broilers, 0.77 L/h/Kg for azithromycin **(Abo-El-Sooud *et al.,* 2012)** but not to that in foals for clarithromycin, 1.27 L/h/Kg **(Womble *et al.,* 2006) .** This finding is in agreement with that of a previous study in mice has shown that fluconazole has the effect of reducing the clearance of antipyrine which is metabolized almost entirely by oxidative metabolism by hepatic cytochrome P450 enzymes (**Bibi, 2008**) .

The elimination half-life ($t_{½\beta}$) was found to be (3.38 ± 0.31 h and 3.61 ± 0.26 h) for clarithromycin and clarithromycin – fluconazole groups respectively . The $t_{½\beta}$ is the time taken for the blood concentration of the drug to decline by 50% during the elimination phase of the disposition curve. This result is shorter than reported in broiler chickens was 4.5 h **(Hanady *et al.,* 2016)** and in foal was 5.4 h **(Womble *et al.* 2006)**. 30% to 40% of an oral dose of clarithromycin is excreted unchanged or as an active metabolite via the kidneys and the remainder is excreted via the bile. Clarithromycin is metabolized by CYP 3A4 to its 14-hydroxy active metabolite, both of which require renal excretion **(Ma *et al.* (2014)**. The half-life of oral clarithromycin was around 2 h after a single dose but increased to approximately 4 h in plasma and tissues after repetitive administration in male volunteers **(Traunmuller *et al.,* 2007)**. The difference in the above values could be due to a difference in the dose, route of administration or animal species.

In the present study, the value of V_{dss} was significantly higher in Group I (2.79 ± 0.11 mg /(µg/ml)) than Group III (2.35 ± 0.16 mg /(µg/ml)). This small volume of distribution in the two groups indicate that the drug mainly confined to the intravascular fluid as the drug binds preferably to plasma proteins (e.g. to albumin) and much less to tissue proteins . In this respect, **Arsic *et al.* (2019)** reported that macrolide antibiotics are known to bind to plasma proteins, particularly alpha1-acid glycoprotein. Nevertheless, this V_{dss} of clarithromycin was still smaller than that reported for clarithromycin in broiler chickens, 6.89 L **(Hanady *et al.,* 2016)** and foal, 10.4 L **(Womble *et al.,* 2006).**

After intramuscular administration, maximum serum concentration (C_{max}), area under the curves (AUC_{0-t}, AUC_{0-inf} ,AUMC) and zero time intercept of the elimination phase (B) were significantly higher in clarithromycin - fluconazole group.

The maximal serum concentrations (C_{max}) is shown to reflect not only the rate but also the extent of absorption. In our study, C_{max} after I.M. administrations were 1.23 ± 0.01 µg/ml and 1.42 ± 0.02 µg/ml with time to peak concentration (T_{max}) values of (1.23 ± 0.01 h) and (1.24 ± 0.01 h) in Group II and Group IV respectively.

The C_{max} is highly correlated with the area under the curve (AUC) contrasting blood concentration with time. The significantly higher

values of AUC $_{0\text{-}t}$ (7.15±0.05 µg/ml.h), AUC $_{0\text{-}inf}$ (7.99±0.10µg/ml.h) and AUMC (42.94 µg/ml.h^2) were observed in clarithromycin - fluconazole group might be attributable to the enhanced absolute availability of clarithromycin .These findings indicate complete absorption of the drug from I.M. Administration.

In this respect, Fluconazole (400 mg on day 1 and 200 mg on days 2 to 4) elevated the C_{max} and $AUC_{(0\text{-}12)}$ of clarithromycin by 12% and 18% in healthy subjects respectively **(Gustavson *et al.,* 1996)**. When rifabutin was given as a two-drug combination with either fluconazole or clarithromycin, the AUC increased 76% compared to that of rifabutin alone **(Jordan *et al.,* 2000) .**

In addition, the prophylactic effect of rifabutin for *Mycobacterium avium* complex bacteremia in AIDS patients is enhanced by concomitant treatment with fluconazole, possibly as a result of higher rifabutin concentration due to inhibitory effect of fluconazole on cytochrome P450 enzyme systems. There have been reports that an interaction exists when fluconazole is administered concomitantly with rifabutin, leading to increased serum levels of rifabutin up to 80%. **(Pfizer, 2018).**

Fluconazole has been shown to be a potent inhibitor of fungal cytochrome P450, and may interfere with hepatic metabolism of several other co-administered medications **(Somchit *et al.,* 2009)**. Fluconazole produced a mean 83% increase in the AUC of S-(+)-ibuprofen , the increase was evident in male volunteers **(Ville-Veikko *et al.*, 2006)** while Fluconazole inhibits the metabolism of losartan to its active metabolite (E-31 74) which is responsible for most of the angiotensin II-receptor antagonism which occurs during treatment with losartan. The C_{max} and AUC of flurbiprofen were increased by 23% and 81%, respectively, when coadministered with fluconazole compared to administration of flurbiprofen alone **(Sandoz, 2018).**

There are a possible interactions between fluconazole and non-steroidal anti-inflammatory drugs (NSAIDs). Treatment with fluconazole significantly increased the AUC of the CYP-2C9 substrate celecoxib. Recently, it has been shown that co-administration of fluconazole and lumiracoxib (novel cyclooxygenase 2 selective inhibitor) caused a small (18%) increase in the mean AUC of lumiracoxib **(Scott *et al .,* 2004)**. In addation, it was found that bioavailability of paroxetine (antidepressant) was increased by itraconazole (antifungal) **(Yasui-Furukori *et al. ,* 2007).**

From the above data, we can explained the effect of fluconazole on pharmacokinetic behavior of clarithromycin, where clarithromycin is metabolized by cytochrome P450 enzymes **(Kaneko *et al.,* 2017)** and the significant effect of fluconazole may be due to the inhibitory affinity of fluconazole for mammalian cytochrome P450 enzyme systems and/or to the metabolic pathways of clarithromycin being primary dependent on the activity of cytochrome P450 enzymes.

The use of antibacterial in animal is often associated with incomplete bacterial eradication, resulting in an insufficient clinical response in some cases and the risk of the emerge of antibacterial resistance **(Haritova *et al.,* 2006).**

The macrolide antimicrobial agents display variable concentration-dependent killing, indicating the increasing importance of the C_{max} parameter. To evaluate the efficacy and the dosing regimen for concentration dependent antibiotic, the ratio C_{max}/MIC, considering that values above 8–10 would lead to better clinical results and to avoidance of bacterial resistance emergence **(Walker, 2000) .**

The susceptible MIC breakpoint of clarithromycin against *Staphylococcus*, *Streptococcus* and *Kingella kingae* were ≤1 mg/L, ≤ 0.25 mg/L and ≤ 0.5 mg/L respectively **(Eucast, 2019)**. Clarithromycin and 14-(R) hydroxyl clarithromycin have a minimum inhibitory concentration (MIC_{90}) of 0.03 and 0.06 µg/ml for H. pylori, respectively **(Ortiz *et al.,* 2007).** An average MIC_{90} of 0.1 µg/ml of clarithromycin has been taken into consideration for calculation of efficacy predictors.

In the present study, C_{max} was 1.23 and

1.42mg/ml in Group II and Group IV, Accordingly C_{max}/MIC ratio was 12.3 and 14.2 respectively indicates potential clinical and bacteriological efficacy. Clarithromycin at dose of 10 mg kg^{-1} b.wt. administered intramuscularly every 12 hrs intervals in rabbits can maintain effective serum concentrations against bacteria with MIC_{90}> 0.1 µg/ml.

No clarithromycin residues were detected in tissues after 24 hrs except in lung, liver and kidney in both groups (Group V and Group VI). No significant difference in serum and tissue residues of clarithromycin between the two groups.

After 24 hrs, the highest concentration was observed in lung (0.31±0.008 ,0.32±0.01 ug/gm) followed by liver (0.28±0.02, 0.26 ±0.01 ug/gm) and kidney (0.23 ± 0.01, 0.22 ± 0.007 ug/gm) while in serum, the clarithromycin concentration were 0.23 ±0.007 ug/ml and 0.20 ±0.008 ug/ml after 24 hrs post injection in both groups (Group V and Group VI) respectively.

Due to Lack of prior research studies on clarithromycin residues in animals, we compare our study with the members of the same macrolide group.

No erythromycin residues were detected in tissues and plasma after 24 hrs except in liver and kidney where it persisted during 48 hrs following intramuscular and oral administrations **(Goudah *et al.,* 2004).** In addition, the maximum residue limit of tylosin in poultry was 100 ug kg^{-1} in muscle, liver and kidney (**EMA, 2002**) and the withdrawal period of 6 days after the last oral administrations was approved **(Ahmed and Mahmoud (2016)**.

Accordingly, a minimum period of 4 days withdrawal between IM injection of clarithromycin in rabbits and slaughter is recommended to be sure that all tissues are free from the drug residues.

Conclusion

Fluconazole can be used effectively along with clarithromycin to treat the drug sensitive microbial infections associated with fungal infection as a favorable pharmacokinetic properties of clarithromycin in co-administration with fluconazole were observed such as improvement in the rate and extent of absorption compared to clarithromycin alone. Clarithromycin should be administered at a dose of 10 mg kg^{-1} b.wt. every 12 hours intramuscularly, to treat bacterial infections with MIC_{90} > 0.1 µg/ml. To ensure delivery of safe animal products to consumers, the recommended withdrawal time for clarithromycin was 4 days in rabbits.

Table (1). Comparative pharmacokinetic parameters of clarithromycin (Mean ± SD) following single intravenous administration of clarithromycin alone and co-administered with Fluconazole (10 mg kg^{-1} b.w., I.M.) in rabbits (n=5).

Parameter	Unit	clarithromycin	Clarithromycin With Fluconazole
A	µg/ml	9.32 ± 0.19	9.15 ± 0.32
α	1/h	2.32 ± 0.20	1.98 ± 0.12*
B	mg/ml	1.83 ± 0.24	2.13 ± 0.20
b	1/h	0.19 ± 0.01	0.20 ± 0.02
K_{ab}	1/h	0.82 ± 0.02	0.75 ± 0.03*
k_{12}	1/h	1.14 ± 0.07	0.89 ± 0.12*
k_{21}	1/h	0.54 ±0.06	0.55 ± 0.08
$t_{0.5\alpha}$	h	0.30 ±0.02	0.34 ± 0.02
$t_{0.5(\beta)}$	h	3.38 ± 0.31	3.61 ± 0.26
	mg/ml	11.15 ± 0.25	11.29 ± 0.23
V_c	(mg)/(µg/ml)	0.89 ± 0.02	0.88 ± 0.01
Cl	(mg)/(µg/ml)/h	0.74 ± 0.01	0.66 ± 0.02*
V_2	(mg)/(µg/ml)	1.89 ±0.11	1.46 ± 0.15*
CL_2	(mg)/(µg/ml)/h	1.02 ± 0.04	0.79 ± 0.09*
AUC_{0-t}	µg/ml.h	12.55 ± 0.12	14.10 ± 0.35
AUC_{0-inf}	µg/ml.h	13.49 ± 0.17	14.99 ± 0.36
AUMC	$\mu g/ml.h^2$	51.01 ± 3.34	53.10 ± 3.55
MRT	h	3.77 ± 0.19	3.53 ± 0.21
V_{dss}	mg/(µg/ml)	2.79 ± 0.11	2.35 ± 0.16*

Values were significantly different at $P<0.05$

A - zero time intercept of the distribution phase, α: Distribution rate constant, B: zero time intercept of the elimination phase, b: Elimination rate constant, k_{ab}: absorption rate constant, k_{el}: elimination rate constant, $t_{0.5\alpha}$: distribution half-life; $t_{0.5\beta}$: elimination half-life; K_{12}: First order transfer rate constant for drug distribution from central to peripheral compartment; K_{21}: First order transfer rate constant for drug distribution from peripheral to central compartment;, Cp^0: serum drug concentration at t=0, V_c: The apparent volume of central compartment; Cl: total body clearance; V_2: The apparent volume of peropheral compartment; CL_2: intercompartmental clearances; AUC_{0-t} : area under the curve; AUC_{0-inf} : area under the curve from zero to infinity; AUMC : is the area under the first moment curve; MRT: mean residence time , V_{dss}: volume of distribution at steady state .

Table (2). Comparative pharmacokinetic parameters of clarithromycin (Mean ± SD) following single intramuscular administration of clarithromycin alone (10 mg kg^{-1} b.w.) and and co-administered with Fluconazole (10 mg kg^{-1} b.w. , I.M.) in rabbits (n=5)

Parameter	Unit	clarithromycin	Clarithromycin with Fluconazole
A	mg/ml	12.93 ± 1.73	13.71 ± 3.86
α	1/h	0.97 ± 0.39	0.97 ± 0.36
B	µg/ml	1.08 ± 0.07	1.29 ± 0.13*
b	1/h	0.17 ± 0.06	0.17 ± 0.07
$t_{0.5\alpha}$	h	0.71 ± 0.03	0.71 ± 0.02
K_{ab}	1/h	1.12 ± 0.04	1.13 ± 0.05
$t_{0.5\ ab}$	h	0.61 ± 0.02	0.60 ± 0.03
k_{el}	1/h	0.37 ± 0.02	0.36 ± 0.02
$t_{0.5(\beta)}$	h	3.94 ± 0.16	3.87 ± 0.24
k_{12}	1/h	0.31 ± 0.01	0.30 ± 0.01
k_{21}	1/h	0.46 ± 0.05	0.47 ± 0.06
V/F	(mg)/(mg/ml)	3.88 ± 0.14	3.39 ± 0.16
Cl/F	(mg)/(mg/ml)/h	1.44 ± 0.02	1.25 ± 0.01
T_{max}	h	1.23 ± 0.01	1.24 ± 0.01
C_{max}	µg/ml	1.23 ± 0.01	1.42 ± 0.02*
AUC_{0-t}	µg/ml.h	6.17 ± 0.07	7.15 ± 0.05*
AUC_{0-inf}	µg/ml.h	6.92 ± 0.11	7.99 ± 0.10*
AUMC	$\mu g/ml.h^2$	37.65 ± 1.33	42.94 ±1.91*
MRT	h	5.43 ± 0.11	5.37 ± 0.18

Values were significantly different at $P<0.05$

A - zero time intercept of the distribution phase, α: Distribution rate constant, B : zero time intercept of the elimination phase, b: Elimination rate constant, k_{ab}: absorption rate constant, k_{el}: elimination rate constant, $t_{0.5\alpha}$:distribution half-life; $t_{0.5\beta}$: elimination half-life; K_{12}: First order transfer rate constant for drug distribution from central to peripheral compartment; K_{21}: First order transfer rate constant for drug distribution from peripheral to central compartment;, Cp^0: serum drug concentration at t=0 , V_c: The apparent volume of central compartment; Cl: total body clearance; V_2: The apparent volume of peropheral compartment; CL_2: intercompartmental clearances; AUC_{0-t} : area under the curve ; AUC_{0-inf} : area under the curve from zero to infinity; AUMC : is the area under the first moment curve; MRT: mean residence time , V_{dss}: volume of distribution at steady state .

Table (3). Serum and tissue concentration (Mean ± SD) of clarithromycin (µg/ml or µg/gm) after dosing of 10 mg kg^{-1} b.wt for five successive days intramuscularly with or without fluconazole (10 mg kg^{-1} , I.M.) in rabbit tissues (n=3).

Tissue and serum	The concentration (µg/gm)					
	1St day		3rd day		5 th day	
	Clarithromy-cin	Clarithromycin with Fluconazole	Clarithromycin	Clarithromycin with Fluconazole	Clarithromy-cin	Clarithromy-cin with Fluconazole
serum	0.23± 0.007	0. 20 ± 0.008	ND	ND	ND	ND
Liver	0.28 ±0.02	0.26±0.01	0.16±0.008	0.17 ±0.01	ND	ND
kidney	0.23± 0.01	0.22±0.007	0.15±0.01	0.16±0.009	ND	ND
Lung	0.31±0.008	0.32± 0.01	0.18±0.01	0.19±0.008	ND	ND
Heart	ND	ND	ND	ND	ND	ND
spleen	ND	ND	ND	ND	ND	ND
Breast muscle	ND	ND	ND	ND	ND	ND

*ND: Not detected

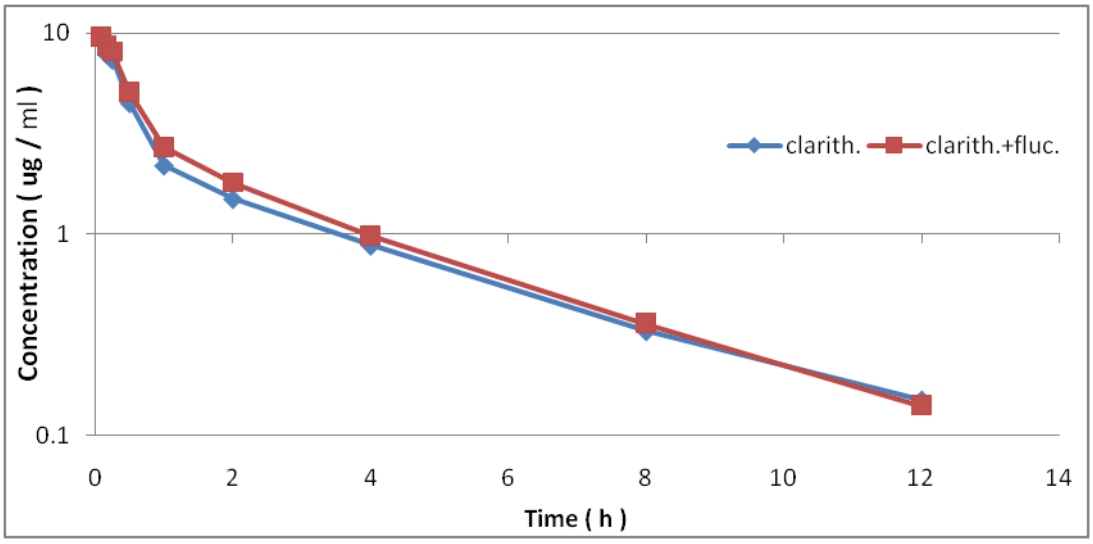

Figure (1). Mean (± S.D.) serum concentrations of clarithromycin following single intravenous administration of clarithromycin alone (10 mg kg^{-1} b.w.) and co-administered with Fluconazole (10 mg kg^{-1} b.w. , I.M.) in rabbits.

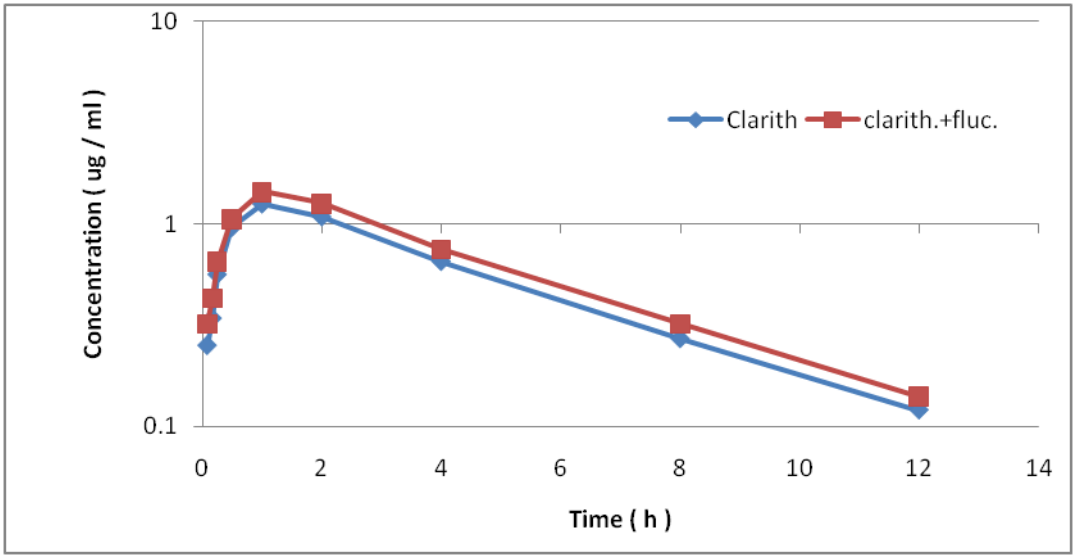

Figure (2). Mean (± S.D.) serum concentrations of clarithromycin following single intramuscular administration of clarithromycin alone (10 mg kg^{-1} b.w.) and co-administered with Fluconazole (10 mg kg^{-1} b.w., I.M.) in rabbits.

Reference

Abo-El-Sooud, K.; Fahmy, E.; Afifi, N. and El-Aty, A.A. (2012). Pharmacokinetics and bioavailability of azithromycin following intramuscular and oral administrations in broiler chickens. Biosciences, 6, 264-270.

Ahmed, M.S. and Mahmoud, S. (2016). Pharmacokinetics and tissue residues of tylosin in broiler . Pharmacology and Pharmacy, 7, 36-42.

Arsic, B.; Novak, P.; Barber, J.; Rimoli, M.G.; Kragol, G. and Sodano, F. (2019). Macrolides: Properties, Synthesis and Applications, 14 (3). DOI: 10. 1002/ cmdc. 201900016.

Aslan, V.; Maden, M.; Erganis, O.; Birdane, F.M. and Corlu, M. (2002). Clinical efficacy of florfenicol in the treatment of calf respiratory tract infections. Veterinary Quarterly, 24: 1.

Belda, B.; Petrovitch, N. and Mathews, K.G. (2018). Sinonasal aspergillosis: Outcome after topical treatment in dogs with cribriform plate lysis. J. Vet. Intern. Med., 32(4): 1353–1358.

Blasi, F. (2004). Atypical pathogens and respiratory tract infections. European Respiratory Journal, 24, 171–181.

Bibi, Z. (2008). Role of cytochrome P450 in drug interactions. Nutr. Metab. (Lond). 5: 27. doi: 10.1186/1743-7075-5-27.

Cyphert, E.L.; Wallat, J.D.; Pokorski, J.K. and Von Recum, H.A. (2017). Erythromycin modification that improves its acidic stability while optimizing it for local drug delivery. Antibiotics 6(11), 1-15.

EL-Sayed, M.G.A.; EL-Komyl A.A.A. and Soliman, A.A. (2018). Pharmacokinetics, bioavilability and tissue residues of apramycin in normal chickens and Escherichia coli infected broiler chickens. World Journal of Pharmacy and Pharmaceutical Sciences, 7 (4), 194-206.

EMA (European medicines agency), (2002). Committee for veterinary medicinal products, tylosin, summary report (5). EMA /MRL /829/02- final , January.

EUCAST (European Committee on Antimicrobial Susceptibility Testing) (2019). Breakpoint tables for interpretation of MICs and zone diameters Version 9.0, 1-99.

Felton, T.; Troke, P.F. and Hope, W.W. (2014). Tissue Penetration of Antifungal Agents . Clinical Microbiology Reviews, 27 (1), 68 – 88.

Garcia-Cuesta, C.; Sarrion-Pérez, M. and

Bagán, J.V. (2014). Current treatment of oral candidiasis: A literature review. J. Clin. Exp. Dent., 6(5): e576-82.

Goins, R.A.; Ascher, D.; Waecker, N.; Arnold, J. and Moorefield, E. (2002). Comparison of fluconazole and nystatin oral suspensions for treatment of oral candidiasis in infants. Pediatr. Infect. Dis. J.; 21: 1165-1167.

Goudah, A.; Abo El Sooud, K. and Abd El-Aty, A.M. (2004). Pharmacokinetics and tissue residue profiles of erythromycin in broiler chickens after different routes of administration. Dtsch Tierarztl Wochenschr., 111 (4): 162-165.

Gustavson, L.E.; Shi, H.; Palmer, R.N. and Carl Craft, J. (1996). Drug interaction between clarithromycin and fluconazole in healthy subjects. Clin. Pharmacol. and Therapeut., 59(2): 185-185.

Hanady, A.; Shaban, A. and Abubakr, E.M. (2016). Pharmacokinetics of clarithromycin after single intravenous and intracrop bolus administrations to broiler chickens .Int. J. of Pharmacol. and Toxicol., 4 (1) , 12-18.

Haritova A.M., Rusenova N.V., Parvanov P.R., Lashev L.D. and Fink-Gremmels J. (2006). Pharmacokinetic – pharmacodynamic modelling of danofloxacin in Turkeys. Vet. Resear. Communicat., 30(7), 775–789.

Haynes, R.R.; Connolly, P.A.; Durkin, M.M.; LeMonte, A.M.; Smedema, M.L.; Brizendine, E. and Wheat, L.J. (2002). Antifungal therapy for central nervous system histoplasmosis, using a newly developed intracranial model of infection. J Infect. Dis., 15;185(12):1830-2

Jacks, S.; Giguère, S.; Gronwall, R.R.; Brown, M.P. and Merritt, K.A. (2002). Disposition of oral clarithromycin in foals. J. Vet. Pharmacol. Ther., 25(5), 359-362.

Jordan, M.K.; Polis, M.A.; Kelly, G.; Narang, P.K.; Masur, H. and Piscitelli, S.C. (2000). Effects of Fluconazole and Clarithromycin on Rifabutin and 25-*O*-Desace-tyl rifabutin Pharmacokinetics. Antimicrob. Agents Chemother., 44(8): 2170–2172.

Kaneko, T.; Arai, M.; Watanabe, A. and Tsuruoka, S. (2017). Effectiveness of measuring genetic polymorphisms in metabolizing enzymes of tacrolimus within one medical facility. J. Nippon Med. Sch.; 84 (6).

Laurie W.L. and Deborah A.H. (2010). Mixed bacterial-fungal infections in the cystic fibrosis (CF) respiratory tract.Med. Mycol., 48 (Suppl. 1), S125–S132.

Lee, A.K.; Lee, J.H.; Kwon, J.W.; Kim, W.B.; Kim. S.G.; Kim, S.H. and Lee, M.G. (2004). Pharmacokinetics of clarithromycin in rats with acute renal failure induced by uranyl nitrate. Biopharm Drug Dispos., 25 (6): 273-282.

Liu, S.; Hou, Y.; Chen, X.; Gao, Y.; Li, H. and Sun, S. (2014). Combination of fluconazole with non-antifungal agents: a promising approach to cope with resistant Candida albicans infections and insight into new antifungal agent discovery. Int. J. Antimicrob. Agents; 43(5), 395–402.

Ma, T.K.; Chow, K.; Choy, A.S.M.; Kwan, B.C.; Szeto, C. and Li, P.K. (2014). Clinical manifestation of macrolide antibiotic toxicity in CKD and dialysis patients. Clin. Kidney J.; 7(6): 507–512.

Mazepa, A.S.; Trepanier, L.A. and Foy, D.S. (2001). Retrospective comparison of the efficacy of fluconazole or itraconazole for the treatment of systemic blastomycosis in dogs. J. Vet. Intern. Med., 25(3): 440-445.

Ortiz, R.A.M.; Calafatti, S.A.; Moraes, L.A.; Deguer, M.; Ecclissato, C.C.; Marchioretto, M.A.M.; Ribeiro, M.L.; Bernasconi, G. and Pedrazzoli, J. (2007). Effect of Helicobacter pylori infection and acid blockade by lansoprazole on clarithromycin bioavailability. Braz. J. Med .Biol. Res., 40 (3), 383-389.

O'Toole, T.E.; Sato. A.F. and Rozanski, E.A. (2003). Cryptococcosis of the central nervous system in a dog. J. Am. Vet. Med. Assoc. 15, 222(12): 1722-5.

Pfizer (2018). Diflucan® (fluconazole), tablets 50 mg and 100 mg . Pfizer Products Inc. Pfizer Canada Inc., Licensee Pfizer Canada Inc., 1-48.

Pennisi, M.G.; Hartmann, K.; Lloret, A.; Ferrer, L.; Addie, D.; Belák, S.; Boucraut-Baralon, C.; Egberink, H.; Frymus, T.; Gruffydd-Jones, T.; Hosie, M.J.; Lutz, H.; Marsilio, F.; Möstl, K.; Radford, A.D.; Thiry, E.; Truyen, U. and Horzinek, M.C. (2013). Cryptococcosis in cats: ABCD guidelines on prevention and management. J. Feline Med. Surg. 15(7): 611-8.

Riddell, J. I.V.; Grant, M.C. and Carol, A.K. (2011). Treatment of Endogenous Fungal Endophthalmitis: Focus on New Antifungal Agents. Reviews of Anti-Infective Agents, 52 (1), 648-653.

Sandoz (2018). Product monograph, fluconazole injection, antifungal agent, solution for intravenous infusion 2 mg/mL. Sandoz Canada Inc., Sandoz Standard, 1- 48.

San Martín, B.; Cornejo, J.; Iragüen, D.; Hidalgo, H. and Anadón, A. (2007). Depletion study of enrofloxacin and its metabolite ciprofloxacin in edible tissues and feathers of white leghorn hens by liquid chromatography coupled with tandem mass spectrometry. J. Food Prot., 70(8), 1952-1957.

Scott, G.; Yih, L.; Yeh, C.M.; Milosavljev, S.; Laurent, A. and Rordorf, C. (2004). Lumiracoxib: pharmacokinetic and pharmacodynamic profile when coadministered with fluconazole in healthy subjects. J. Clin. Pharmacol. 44: 193-199.

Snedecor, G.W. and Cochran, W.G. (2014). Statistical Methods 8th ed. Iowa State Univ., Press Amer., USA . J. of Educ. and Behavior Statistics, 40 , No., 6.

Somchit, N.; Ngee, C.S.; Yaakob, A.; Ahmad, Z. and Zakaria, Z.A. (2009). Effects of Cytochrome P450 Inhibitors on Itraconazole and Fluconazole Induced Cytotoxicity in Hepatocytes. J. Toxicol., Article ID 912320, 1-7.

Steeve, G. (2010). Therapy of Rhodococcus equi Infections in Foals. AAEP Proceedings Vol. 56.

Traunmuller, F.; Zeitlinger, M.; Zeleny, P.; Muller, M. and Joukhadar, C. (2007). Pharmacokinetics of Single and Multiple-Dose Oral Clarithromycin in Soft Tissues Determined by Microdialysis. Antimicrobial Agents And Chemotherapy, 51(9), 3185–3189.

Ville-Veikko, H.; Klaus, T.O.; Kari, L.; Stefan, L.; Pertti, J.N.; Anders, R.; Mika, V.; Hanna, V. and Kari, L. (2006). Effects of the Antifungals Voriconazole and Fluconazole on the Pharmacokinetics of S-(+)- and R-(−)-Ibuprofen. Antimicrob. Agents Chemother; 50(6): 1967–1972.

Walker, R.D. (2000). The use of fluoroquinolones for companion animal antimicrobial therapy. Austral. Veter. J., 78(2), 84–90.

Womble, A.Y.; Giguère, S.; Lee, E.A. and Vickroy, T.W. (2006). Pharmacokinetics of clarithromycin and concentrations in body fluids and bronchoalveolar cells of foals. Americ. J. of Vet. Resear., 67, 1681 -1686.

Yasui-Furukori, N.; Saito, M.; Niioka, T.; Inoue, Y.; Sato, Y. and Kaneko, S. (2007). Effect of itraconazole on pharmacokinetics of paroxetine: the role of gut transporters. Ther. Drug Monit.; 29(1): 45-8.

Zhang, Y.; Huo, M.; Zhou, J. and Xie, S. (2010). PK Solver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Computer methods and programs in biomedicine; 99 (3): 306-314.