ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(2):286-290

ORIGINAL RESEARCH ARTICLE

Pharmacokinetics Modeling and Simulation of Ofloxacin in Oryctolagus Cuniculus Male Rabbits

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Received 21 Nov 2011; Revised 29 Feb 2012; Accepted 11 Mar 2012

ABSTRACT

The pharmacokinetic of oral ofloxacin has been reported extensively in volunteers, very limited animal data are reported in the literature and parameters such as bioavailability in rodents are unknown. Hence, the present study aims to investigate the pharmacokinetics parameters of ofloxacin in oryctolagus cuniculus rabbits after intravenous (10 mg/kg) and oral (20 mg/kg) administration. The experimental data were adequately fitted to a two-compartment model after intravenous and a one compartment model with first order absorption after oral dosing. The total clearance, terminal half-life and apparent volume of distribution were statistically similar after intravenous and oral administration, by both model independent and compartmental approaches. The area under the curve was reduced after oral dosing in comparison to intravenous dosing leading to an oral bioavailability of 63.08 ± 6.98 %. The absorption was fast, with a constant absorption rate of 10.0 ± 3.6 h–1. The results evidenced the linear pharmacokinetics of ofloxacin in rabbits in the dose range of 10 to 20 mg/kg.

Key words: *Ofloxacin, first order absorption, two-compartment model and oral bioavailability*

INTRODUCTION

Oflaxacin has been proposed as a treatment option for a variety of bacterial infections, including Community-acquired respiratory tract infections, urinary tract infections, and skin or skin structure infections [1] due to its extended *in vitro* spectrum of activity, favorable pharmacokinetics Characteristics, and lower potential for drug resistance. Similar to several other fluoroquinolones, Ofloxacin has enhanced potency against Gram-positive cocci, including multiple-drug-resistant *Streptococcus pneumoniae* isolates [2]. Although the pharmacokinetic of oral ofloxacin has been studied extensively in volunteers [3-10], very limited animal data are reported in the literature and parameters such as bioavailability in rodents are unknown. The knowledge of rodent's kinetics is important when evaluating antimicrobial tissue penetration in healthy and infected animals viewing to develop a mathematical model to relate antimicrobial effect (pharmacodynamic-PD) and its biophase concentrations (pharmacokinetics-PK) in a PK/PD model [11]. This investigational approach in animals, even when the drug has already been used in humans, can support dosing regimen evaluation in order to increase the likelihood of clinical success. The results from pre-clinical studies can also allow the investigation of other drug applications not currently used in humans. In this context, the objective of this study was to determine the pharmacokinetic plasma parameters of ofloxacin in Oryctolagus Cuniculus Male Rabbit after intravenous and oral administration.

MATERIALS AND METHODS

Chemicals and reagents

Ofloxacin and norfloxacin (Sigma–Aldrich Co, St Louis, MO, USA) were obtained as gift samples from department of pharmacology, college of medicine, Dankook University, Cheonan, South Korea. Methanol and acetonitrile grade were pursed from Triveni interchem P.Ltd (India). Formic acid AR was pursed from Manav Biochem Impex P.Ltd (India). Reagent grade triple deionized water was pursed from Organo Biotech Laboratories. P.Ltd (India). All other chemicals and reagents used were of analytical grade.

Apparatus and conditions

The LC-MS/MS system used was a Varian ProStar™ LC unit (Varian Inc., CA, USA) connected to a Varian 1200L quadruple. System

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control and data analysis were carried out using Varian MS software (Version 6.5, Varian Inc.). HPLC columns YMC® C18 (Waters, MO, USA), 50 mm × 2.0 mm, 3 µm particle size and guard column (C18, 4.0 X 2.0mm, phenomenex, CA, USA) were used for analyzing blood samples. An isocratic mobile phase consisting of solvent A (purified water containing 0.1% acetic acid) and solvent B (acetronitrile containing 0.1% acetic acid) mixed in the ratio of 60/40 (v/v, A/B) was used at a flow rate of 0.21 ml/min.The column oven was maintained at 50ºC and run time was 5 minutes. MRM (multiple reaction monitoring) transitions with collision energies (eV) for ofloxacin and norfloxacin were m/z 417.5 → 234 (10.5 eV) and 377.5→ 234 (12.0 eV), respectively. The scan time and dwell time were 0.3 sec and 0.5 sec respectively. Electrospray ionization (ESI) was performed under capillary 5000 volts, shield 600 volts, and at a temperature of 350°C. Manifold temperature and pressure were 41.9°C and 1.83 mTorr, and the detector was set at 1500 volts, fixed positive.

Animal handling and surgical procedures

2.8-3.0 kg Oryctolagus cuniculus male rabbits were pursed from Local animal suppliers, Bharatpur, Chitwan, Nepal. The rabbits were acclimated for one week before study. Upon arrival, animals were randomized and housed one per cage in strictly controlled environmental condition of 20 to 22^{o}C temperature and 50 to 60 % relative humidity (RH). A 12 hour light and dark cycle was used. All animal procedures were based on a guideline recommended by institutional animal care and experiment committee of Chitwan Medical College. The pharmacokinetic evaluation of ofloxacin was conducted using two groups of animals. One group (n = 6) received a single intravenous bolus dose of ofloxacin (10 mg/kg) injected into the lateral tail vein. The second group received a single dose of 20 mg/kg of ofloxacin by oral gavage (n = 6). Ofloxacin solution for intravenous (i.v.) and oral administration was prepared in 0.9% NaCl solution. For plasma sampling rabbits were anesthetized with ketamine (80m g/kg, i.p.) and a cannula was inserted into the carotid artery for blood sampling. At predetermined times points before (zero time) and after dosing (0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 9 h) blood was withdrawn into heparinized centrifuging tubes. The same procedure was carried out after oral administration with sampling at 0, 0.33, 0.66, 1, 1.5, 2, 4, 6, 8 and 10 h. The volume of blood withdrawn was approximately 200 µl per sampling. Plasma was separated by centrifugation at 6800 g, 25 ± 1 °C for 15 min and stored at -4 °C until analysis.

Sample preparation and validation

Blood samples-validation

The validation samples were prepared by standard working solution spiking method to access the plasma concentration of ofloxacin. For the measurement of ofloxacin in plasma sample, the validation samples were prepared by following way; an aliquot of blood plasma 90µL was spiked with 10 µl standard working solution (desirable concentration of ofloxacin standard solution was prepared by dissolving appropriate amount in purified water) and 20 µl internal standard (Norfloxacin,1 µg/ml, prepared in methanol/water, 50/50 v/v), and extracted with 400 µl acetronitrile solution. The organic layer was dried under the gentle stream of nitrogen 40°C. The dried extract was reconstituted with 800 µl of 50% methanol and 5 µl was injected to LC-MS/MS system.

Lower limit of detection (LLOD) was defined as a peak with signal noise ratio(S/N) more than 10/1, while lower limit of quantification was further narrowed to have percentage coefficient of variation (CV, %) less than 15%. Five sets of validation samples at concentrations of 0.1, 0.3, 1, 2, 5, 10, 50 and 100µg/ml were used to draw calibration curve. Similarly, Inter/ Intra- day validation were assessed to validate the precision and accuracy of the assay. For interday validation, five sets of control samples at different concentrations of 0.1, 0.3, 2 and 8µg/ml were evaluated on five different days. For intraday validation, five sets of control samples at different concentrations of 0.1, 0.3, 2 and 8µg/ml with one standard curve were evaluated on same day. The assay recovery for ofloxacin was assessed with five sets of quality control (QC) samples (1, 5 and 10 µg/ml) assayed randomly along with standard samples during the interday and intraday assays.

Blood samples- analysis

Sample preparation involved a protein precipitation method with acetronitrile. An aliquot of blood plasma 100µL was spiked 20 µl internal standard (1 µg/ml, prepared in methanol/water, 50/50 v/v), and extracted with 400 µl acetronitrile solution. The organic layer was dried under the gentle stream of nitrogen 40°C. The dried extract was reconstituted with 800 µl of 50% methanol and 5 µl was injected to LC-MS/MS system.

Data Analysis

Noncompartmental and compartmental pharmacokinetics analyses were performed using winNonlin™ Professional (Version 2.1, Pharsight,

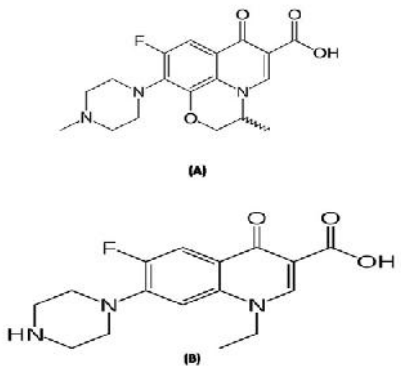
CA, USA). The pharmacokinetic parameters determined by non-compartmental and compartmental analysis for both doses and administration routes investigated were compared by Student *"t"* test (p = 0.05).

RESULTS AND DISCUSSION

Quantitative basis and the selection of internal Standard

In order to develop an analytical method with desired LLOD (100ng/ml), it was necessary to use MS/MS detection, because MS/MS analytical methods provide the very low limits of detection (LOD) required for trace mixture analysis. The internal standard (IS) used norfloxacin (**Fig 1b**) is a structural isomer of ofloxacin (**Fig 1a**). The full scan positive mass spectra of ofloxacin and the IS produced protonated mass ions ($[M+H]^+$) at 417.5 and 377.5, respectively, in the Q1 spectrum, and these were used as precursor ions to obtain product ion spectra. Although both ofloxacin and IS have the same molecular weights, they can be individually detected due to their different fragmentation patterns. No interference was observed between ofloxacin and IS when measuring the m/z 417.5 → 234.0 transition and m/z 377.5 → 234 transition, respectively.

Figure 1:

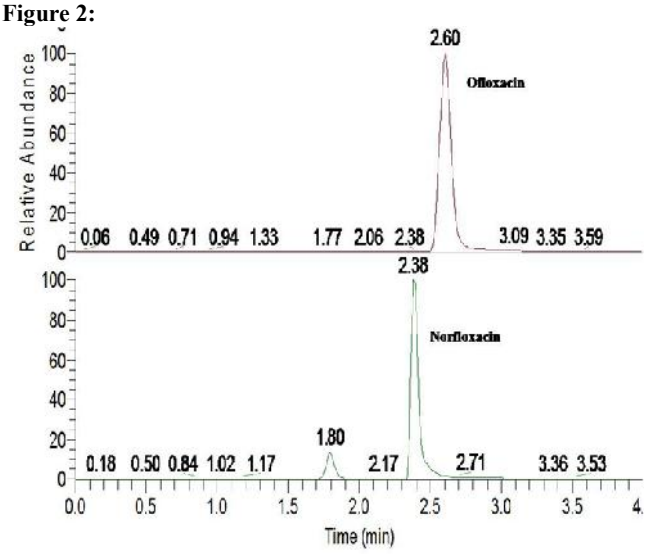


Validation

Chromatographic conditions, especially the composition of the mobile phase, were optimized to achieve good resolution and symmetrical peak shapes for ofloxacin and the IS, acceptable retention factors (k'≥2), and a short run time. The isocratic mobile phase consisting of solvent A (purified water containing 0.1% acetic acid) and solvent B (acetonitrile containing 0.1% acetic acid) mixed in the ratio 60/40 (v/v, A/B) was found to be suitable. A flow rate of 0.21 ml/min was required to elude the ofloxacin and the IS at retention times of 2.60 and 2.38 min, respectively. The acetic acid was found to be necessary in order to lower the pH and protonate ofloxacin to produce a symmetrical peak shape at a satisfactory retention factor. The percentage of acetic acid was also optimized to achieve a symmetrical peak shape and good ionization and fragmentation.

The calibration curve drawn for ofloxacin in plasma for the manual method was linear over the concentration range 0.1 to 100 µg/ml. The best linear fit and least squares residuals of the calibration curve were achieved using a $1/x^2$ weighing factor, giving a mean linear regression equation for the calibration curve of $y=0.78241x + 0.001196$ where y is the peak ratio of ofloxacin to IS and x is the concentration of the ofloxacin. The correlation coefficient (r^2) for ofloxacin was 0.99801. The inter-and intra-day precisions were expressed as CV % and were below 15% (maximum 11.24% and minimum 5.32% for an LLOD sample), and the accuracy was between 98.36% and 107.93%, which complies with the FDA regulations. The recovery percentages of QC samples were between 96.35% and 101.52%. The extraction procedure showed good sensitivity, specificity, precision, accuracy, recovery, and linearity, and hence the method was successfully implemented for the analysis of blood samples. The mass chromatogram of blood samples at 120 min (oral study) is shown in (**Fig 2**).

Figure 2:



Pharmacokinetics testing

The experimental data obtained after pharmacokinetics testing were adequately fitted to a two-compartment model after intravenous dosing resulting in model selection criterion (MSC) values ranging from 6.20 to 8.78 and correlation coefficients from 0.988 to 0.997, showing a good agreement between the experimental data and the model selected. Regarding oral administration, one-compartment model with first order absorption was more appropriate to describe the data, with MSC values ranging from 2.78 to 6.08 and correlation coefficients from 0.921 to 0.990.A result

indicating that the compound has a distinct distribution phase into tissues with a more limited elimination independently of the dose considered. The mean pharmacokinetic parameters obtained after i.v. and oral administration are summarized in (**Table 2 & Table 3**).

There was no statistical difference between the PK parameters determined by compartmental and non-compartmental approaches. In the same manner, the total clearance (CLtot), the terminal half-life (t1/2) and the apparent volume of distribution (Vdss) were statistically similar after i.v. and oral administration. The terminal half life obtained after i.v. and oral dosing by non compartmental analysis were 6.6 ± 1.6 h and 7.4 ± 0.6 h, respectively demonstrating that ofloxacin concentrations decline faster in rabbits. The area under the curve was reduced when the oral route was used, from 13.2 ± 2.6 µg·h/mL after i.v. dosing (10 mg/kg) to 8.2 ± 3.2 µg·h/mL after oral administration (20 mg/kg), leading to an oral bioavailability of 63.080%. The absorption phase was rapid after oral dosing resulting in an absorption rate constant (ka) of 10.0 ± 3.6 h^{-1}. As the peak plasma concentration (Cmax) took place approximately 30 min after the 20 mg/kg oral dosing, only one sample before peak level was collected, making intricate the accurate determination of the absorption rate constant for this dose. When the 10 mg/kg oral dose was previously evaluated, the peak plama level took place 90 min after dosing, allowing a better determination of the absorption rate constant. The comparisons of ofloxacin pharmacokinetic parameters reported in the present paper allow the conclusion that the drug presents linear pharmacokinetics in rodents in the dose range from 10 to 20 mg/kg. Besides the oral bioavailability of ofloxacin in rodents be approximately 1/3 of that reported for humans [4,5] its faster elimination in rabbit lead to a smaller total drug exposition which probably will influence the bactericidal activity of this concentration dependent antimicrobial agent in animals.

ACKNOWLEDGEMENT

We would like to thank Summy Pharmaceuticals P.Ltd, Nawalparasi, Nepal for providing research facilities. We would like to express our sincere thank to Dr.Tae-Ho Kim and Dr. S.Y.Oh of Biomedical Research Center, Chungnam-si, Cheonan, South Korea for their support in analyzing plasma samples using LC-MS/MS.

Table 1: Validation of the LC-MS/MS method for measuring ofloxacin in rabbit plasma

Parameters	Obtained Results
Lower limit of detection (µg/ml)	0.1 µg/ml
Calibration range (µg/ml)	0.1-100 µg/ml
Calibration equation	y = 0.78241x + 0.001196
Coefficient if regression(r^2)	0.99801
Interday Precision	
(CV %,n=5) [a]	10.28
0.1 µg/ml	6.21
0.3 µg/ml	7.87
2 µg/ml	5.91
8 µg/ml	
Interday Accuracy (%,n=5) [b]	
0.1 µg/ml	107.93
0.3 µg/ml	93.79
2 µg/ml	102.36
8 µg/ml	102.98
Intraday Precision	
0.1 µg/ml	11.24
0.3 µg/ml	5.32
2 µg/ml	6.32
8 µg/ml	7.89
Intraday Accuracy (%,n=5) [b]	
0.1 µg/ml	98.36
0.3 µg/ml	108.65
2 µg/ml	120.36
8 µg/ml	100.25
QC samples recovery (%,n=5)	
1 µg/ml	101.52
5 µg/ml	96.35
10 µg/ml	97.82

[a] %CV=Standard deviation of concentrations determined x 100/ Mean concentration determined

[b] Accuracy=Mean concentration determined x100/Concentration expected,

The intra-and inter-day precisions expressed as coefficient of variations percent (% CV) should not exceed 15% at any concentration level, with the exception of LLOD, QC samples, where should not exceed ±20% (Bioanalytical Method Validation, FDA guidelines, May 2001).

Table 2: Ofloxacin pharmacokinetic parameters after single intravenous administration (10mg/kg, n=6) to rabbits

Parameters	Model Independent	Two-Compartment
A (µg/mL)	-	6.6±1.8
B (µg/mL)	-	2.6±1.0
α (h^{-1})	-	5.4±3.4
β or k_e (h^{-1})**	0.44±0.01	0.56±0.1
k_a (h^{-1})	-	-
$t^{1/2}_{elim}$ (h)	6.6±1.6	5.6±1.8
$AUC_{0-\infty}$	13.2±2.6	12.6±2.4
C_{max} (µg/mL)	-	-
T_{max} (h)	-	-
CL_{tot} (L/h/kg)	1.8±0.4	2.0±0.4
V_c (L/kg)	-	2.8±0.6
Vd_{ss} (L/kg)	5.6±0.8	6.0±1.2
MRT (h)	6.2±1.8	-
f (%)	-	-

**Statistical difference in comparison to i.v. parameter ($p<0.05$). **β for two-compartment, k_e for model independent and one-compartment.*

Table 3: Ofloxacin pharmacokinetic parameters after single oral administration (20mg/kg, n=6) to rabbits

Parameters	Model Independent	One-Compartment
A (µg/mL)	-	-
B (µg/mL)	-	-
α (h^{-1})	-	-
β or k_e (h^{-1})**	0.38±0.02	0.44±0.08
k_a (h^{-1})	-	10.0±3.6
$t^{1/2}_{elim}$ (h)	7.4±0.6	6.4±1.2
$AUC_{0-\infty}$ (µg.h/mL)	8.2±3.2*	7.4±2.4*
C_{max} (µg/mL)	1.72±0.64	1.4±0.38
T_{max} (h)	1.1±0.54	1.42±0.48
CL_{tot} (L/h/kg)	2.0±0.6	2.2±0.6
V_c (L/kg)	-	-
Vd_{ss} (L/kg)	6.2±2.0	9.8±3.4
MRT (h)	73.0±1.0	-
f (%)	63.08	-

**Statistical difference in comparison to i.v. parameter ($p<0.05$). **β for two-compartment, k_e for model independent and one-compartment.*

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