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Population Pharmacokinetics Modeling of Levofloxacin in Rabbit After Intravenous Bolus Injection and Peroral Administration

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ABSTRACT

The population-based approach has been widely applied to describe the pharmacokinetic profile of many drugs. This current research aimed to study the population-based pharmacokinetics of levofloxacin in rabbits after intravenous bolus injection and peroral administration. Modeling analyses were performed using Monolix, one of the alternative tools for the population-based approach. Monolix works based on the Stochastic Approximation Expectation-Maximization (SAEM) method. The analysis was performed based on the population model using one-compartmental and two-compartmental disposition models. The combination error model was used during the analyses. Modeling appropriateness was determined based on the goodness of fit analyses, i.e., 1) the individual fit, 2) the observed versus population prediction values; and 3) the observed versus individual prediction values. Plasma concentration profiles of levofloxacin by intravenous bolus injection and peroral administration are better described by an appropriate model using a two-compartmental disposition model. All goodness of fit analyses demonstrates the power of the chosen model. However, the estimated disposition parameter values obtained based on the intravenous bolus injection and peroral administration are different for each subject. To confirm this phenomenon, we performed a simultaneous fitting of all intravenous bolus as well as peroral administration data. The goodness of fit analyses indicates a good fitting of all data.

Keywords: Monolix, intravenous bolus, peroral, the goodness of fit

INTRODUCTION

Levofloxacin is a broad-spectrum fluoroquinolone antibiotic, plays an essential role in the treatment of microbial infections. It has been known that this compound is more active than previous fluoroquinolone generations, i.e., ciprofloxacin (Fu *et al*., 1992). Levofloxacin is reported to be effective against pneumococci, with greater efficacy than ciprofloxacin. This antibiotic is also used to treat bacterial infections of the skin, sinuses, kidneys, bladder, or prostate. Levofloxacin has been reported to be effective in eradicating *Escherichia coli*, a major pathogen causing urinary tract infections (McGregor *et al*., 2009).

Population-based compartment modeling has been developed since the 1970s until now. One of the advantages of the population-based compartment modeling approach is the focus of analysis on the whole population instead of the conventional method based on the two-stage approach based on the parameter values in each subject. The benefit of this condition is the completeness of information retrieval related to the absorption, elimination and/or distribution kinetics is contributed by the whole population (Mold and Upton, 2012; Sheiner *et al*., 1972).

Compartmental modeling uses the assumption that changes in the magnitude of a variable (e.g., concentration or amount of mass to

time) are due to the movement of matter from one area to another. The compartment is defined as the material characteristics that can be in the form of a specific chemical form, biological material (organs, parts of organs) occupying a particular room or volume (Wastney, 1999).

The pharmacokinetics of levofloxacin have been examined in several reports (Cheng *et al*., 2002; Furlanut *et al*., 2003; Ghimire *et al*., 2016). Some of them also implemented the population-based approach to estimate the model parameters. However, none of those studies was performed to find the best model based on a comparison of several approaches. In the present study, we focused on the method using Monolix, one of the alternative tools in population-based analyses (Chan *et al*., 2011; Dartois *et al*., 2007). We also performed pharmacokinetic studies in rabbits, considering the practicality and ease of handling the laboratory animals in blood sample collections.

MATERIALS AND METHODS

Levofloxacin hydrochloride (pharmaceutical grade) was purchased from PT. Kimia Farma Tbk (Bandung, Indonesia), whereas levofloxacin and ciprofloxacin (analytical grade) from Sigma-Aldrich (Buchs, Switzerland). Potassium dihydrogen phosphate, glacial acetic acid, phosphoric acid, acetonitrile, and methanol were of analytical grade and purchased from Merck (Darmstadt, Germany).

Method

Preparation of intravenous bolus and peroral levofloxacin solutions.

Levofloxacin solution was prepared at a concentration of 30mg/mL in 0.9% NaCl. The mixture was vortexed for 1min and sonicated for 5min to obtain a homogeneous solution. The injection volume for intravenous bolus and peroral delivery in the rabbit was 1.5mL and 2mL, respectively.

Pharmacokinetic studies of levofloxacin in rabbits.

Six New Zealand white rabbits (the average weight of 2.9±0.5kg) were divided into two groups. The first group was received 45mg levofloxacin dose as an intravenous bolus injection in the first week, followed by 60mg of levofloxacin via a peroral route in the second week, and vice versa for the second group. Blood samples (1mL) were taken from the rabbit ear vein at 0min; 10; 20; 30; 60; 120; 180; 300; and 480 after treatment and placed into Eppendorf tubes containing heparin. Samples were centrifuged to separate plasma. Plasma samples were then stored frozen (-20˚C) until analysis. The study protocols were approved by the Laboratory Animal Ethics Commission of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Levofloxacin analysis

A total of 300µL plasma was put into 1.5mL Eppendorf tubes, followed by the addition of 50µL of 20ppm standard ciprofloxacin and 800µL acetonitrile. The mixtures were then centrifuged at 5,00 rpm for 10min at 4°C. The supernatant was taken prior to filling into the vial. A total of 800µL acetonitrile was added back to the sludge, and the extraction process was repeated. This later supernatant was mixed with the first one, followed by a solvent evaporation step. Residues were stored at -20°C until analysis. On the day of analysis, the residue was added with 1mL of the mobile phase followed by a mechanical shaking for 1min. Before injection into the HPLC (injection volume 20µL), the solution was filtered using a 0.45µm membrane filter.

Liquid chromatographic system

Levofloxacin analysis was performed by using HPLC L-2000 Hitachi equipped with L-2130 pump, L-2200 autosampler, L2420 UV-Vis detector controlled by D-2000 HSM Elite software with a stationary phase of LiCrosphore® C18 column (length 250 mm; i.d. 4.6mm; particle size 5µm) and a mobile phase consisting of acetonitrile: methanol: 25mM pH 3.0 phosphate buffer (13: 7: 80), pumped at a flow rate of 1.5 mL/min. Levofloxacin detection was performed using an ultraviolet detector at a wavelength of 280nm.

Data analysis

Monolix (Frame, 2006), stand-alone version 2019R2 and running under Windows 10 machine, was used to analyze the data. Firstly, we implemented a population model with one compartment disposition model, followed by the same model but with a two-compartment model. Analyses were performed using the available template models provided by Monolix. The structural model of intravenous bolus injection with the one-compartment disposition model has the elimination rate constant (K) and the distribution volume (Vd) parameters.

The model of peroral administration with the one-compartment model has the absorption rate constant (Ka), K, and Vd parameters.

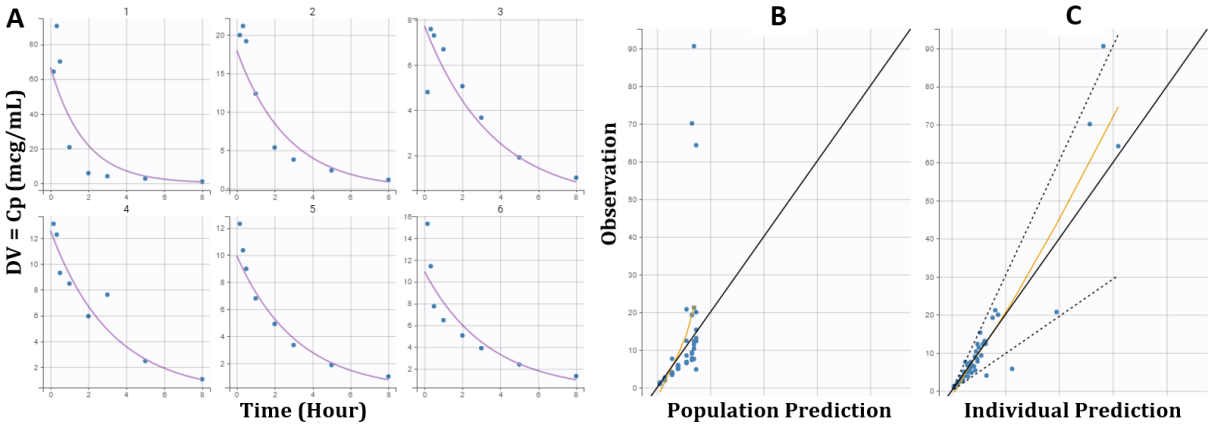

Figure 1. The goodness of fit of the one-compartment open model of levofloxacin after intravenous bolus injection, i.e., individual data fitting analyses (panel A); observation versus population prediction analysis (panel B) and observation versus individual prediction analysis (panel C).

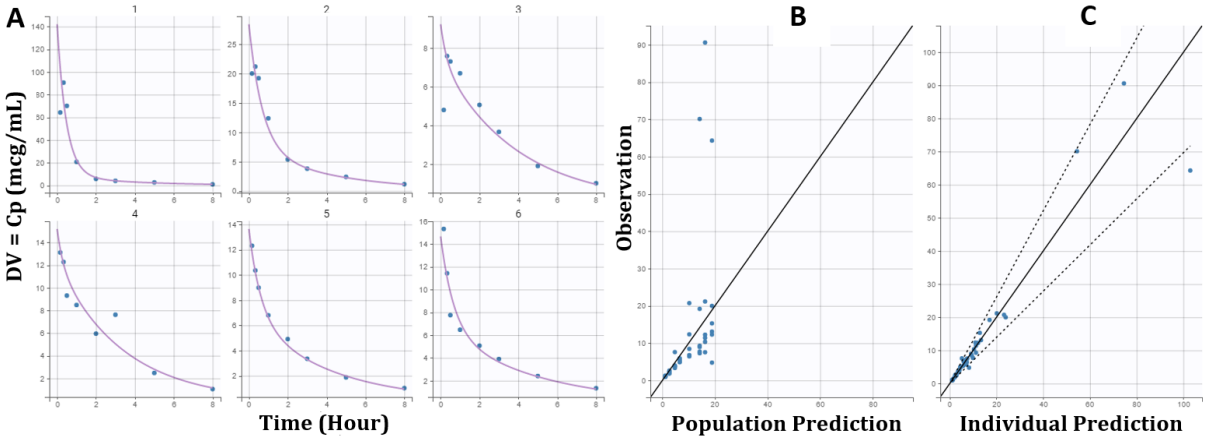

Figure 2. The goodness of fit of a two-compartment open model of levofloxacin after intravenous bolus injection, i.e., individual data fitting analyses (panel A); observation versus population prediction analysis (panel B) and observation versus individual prediction analysis (panel C), with a combination error model implemented.

The structural model of intravenous bolus injection with the two-compartment model has a distribution to the peripheral rate constant (K_{12}), the distribution to the plasma rate constant (K_{21}), K, and Vd parameters. The model for peroral administration with the two-compartment model has the Ka, K_{12}, K_{21}, K, and Vd parameters. No covariate was applied while the covariance implemented the default diagonal pattern.

The adequacy of modeling in all cases was analyzed based on the goodness-of-fit evaluations. These evaluations consisted of 1) the individual fitting with the individual and population model prediction curves; 2) the correlation of DV, namely the dependent variable (the observed Cp) versus population model prediction of Cp; and 3) the correlation of DV versus the individual model prediction of Cp. Such evaluations are considered crucial to judge the adequacy of specific modeling analyses (Mohammed *et al.*, 2012; Owen and Fiedler-Kelly, 2014; Zheng *et al.*, 2014).

RESULTS AND DISCUSSION

Analyses of pharmacokinetic profiles after intravenous bolus injection

Population-based analyses of levofloxacin administration via a bolus injection (Figure 1 (one-compartment open model) and Figure 2 (two-compartment open model)). As demonstrated in those two figures, it can be concluded that the population model with a two-compartment open model can better describe the data.

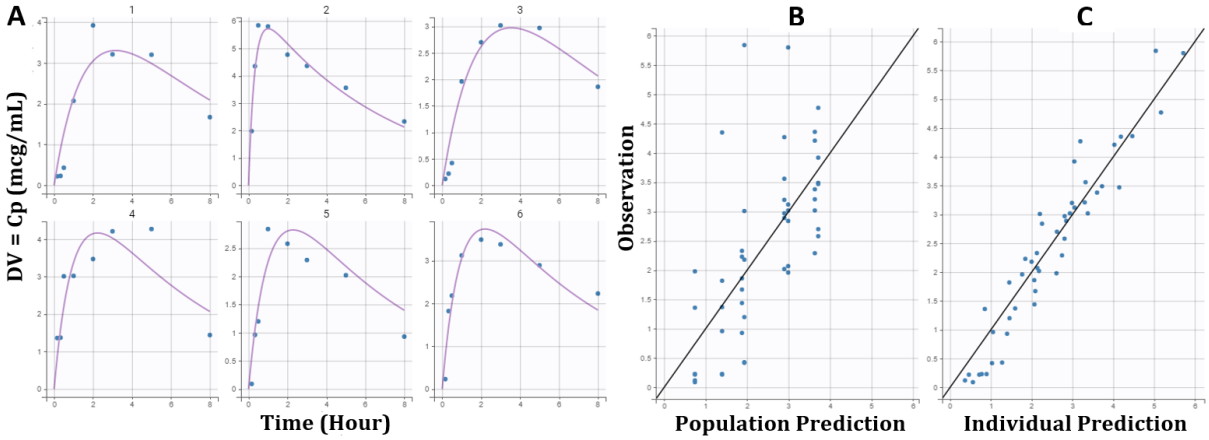

Figure 3. The goodness of fit of the one-compartment open model of levofloxacin after peroral administration, i.e., individual data fitting analyses (panel A); observation versus population prediction analysis (panel B), and observation versus individual prediction analysis (panel C).

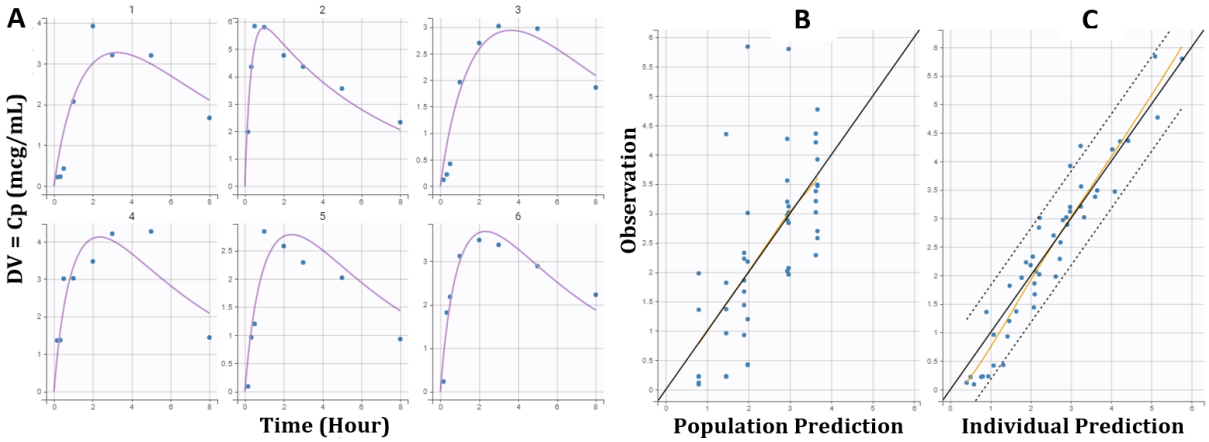

Figure 4. The goodness of fit of the two-compartment open model of levofloxacin after peroral administration, i.e., individual data fitting analyses (panel A); observation versus population prediction analysis (panel B) and observation versus individual prediction analysis (panel C), with a combination error model implemented.

It is clearly shown that the one-compartment model is unable to describe some points of the Cp versus time data.

Analyses of pharmacokinetic profiles following peroral administration

Population-based analyses of levofloxacin administration via peroral administration (Figure 3 and Figure 4) by one- and two-compartment open models, respectively.

Simultaneous fitting of intravenous bolus and peroral administration

Although the fitting with the two-compartment model has described well pharmacokinetic data of intravenous bolus and peroral administration, there is a lack of model parameters estimation. Inconsistency of the disposition parameter estimates following both routes of administration (Table I). In order to overcome this, simultaneous analysis of the data by combining all data in one modeling analysis was performed (Figure 5).

As can be seen from the figures, the simultaneous fitting provides better goodness of fit while maintaining the disposition values parameters in each subject is the same (Table II). Moreover, the individual estimated values of K and Vd were relatively close to the previously published data of levofloxacin pharmacokinetics in rabbits.

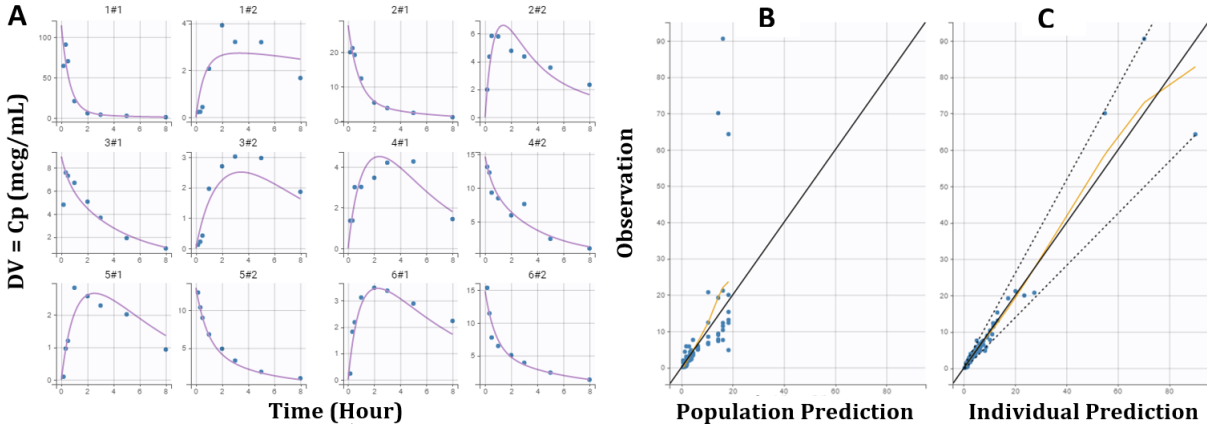

Figure 5. The goodness of fit of a two-compartment open model after a simultaneous fitting of intravenous bolus injection and peroral administration of levofloxacin, i.e., individual data fitting analyses (panel A); observation versus population prediction analysis (panel B) and observation versus individual prediction analysis (panel C), with a combination error model implemented.

Table I. The estimated individual values of levofloxacin pharmacokinetic parameters following intravenous bolus injection and peroral administrations.

ID	Vd (L)		K (h^{-1})		K_{12} (h^{-1})		K_{21} (h^{-1})		Ka (h^{-1})	
	IV	ORAL	IV	ORAL	IV	ORAL	IV	ORAL	IV	ORAL
1	0.324	8.66	1.45	0.201	0.515	0.562	0.358	2.43	NA	0.505
2	1.59	7.03	0.583	0.197	0.514	0.58	0.496	2.25	NA	2.48
3	4.88	8.94	0.308	0.201	0.518	0.578	3.45	2.35	NA	0.419
4	2.99	7.89	0.348	0.199	0.517	0.573	3.64	2.37	NA	0.844
5	3.3	11.33	0.415	0.205	0.519	0.586	1.04	2.23	NA	0.802
6	3.06	8.71	0.377	0.199	0.526	0.588	0.828	2.24	NA	0.842

Legend: NA: not available

Table II. The estimated parameters values of levofloxacin pharmacokinetics after a simultaneous analysis of intravenous bolus injection and peroral administration data.

ID	f	Ka (h^{-1})	Vd (L)	K (h^{-1})	K_{12} (h^{-1})	K_{21} (h^{-1})
1	0.588	0.048	0.397	1.14	0.378	0.274
2	0.593	0.665	1.65	0.513	0.493	0.383
3	0.595	0.328	5.03	0.275	0.303	1.7
4	0.587	0.542	3.1	0.331	0.357	1.63
5	0.584	0.348	3.52	0.412	0.36	0.726
6	0.591	0.449	3.07	0.354	0.49	0.65
Mean*	0.59	0.397	2.795	0.504	0.397	0.894
SD**	0.004	0.212	1.597	0.322	0.077	0.62
POP Value#	0.588	0.304	2.16	0.439	0.354	0.575
Omega##	0.0425	0.875	0.836	0.498	0.474	0.86
a**#				0.249		
b#**				0.173		

the standard deviation of the interindividual variability of pharmacokinetic parameters estimated using Monolix; **# the proportional error estimated using Monolix; ##* the additive error estimated using Monolix

standard deviation of the interindividual variability of Vd and K at 2.16 L and 0.439 h^{-1}, as well as 0.836 and 0.498, respectively (Table 2).

We calculated using Monolix the approximate values of Vd and K from the mean plasma concentration of levofloxacin time profiles data reported by Sitovs *et al*. (2020) at a value of 2.35 L and 1.04 h^{-1}, respectively. Those parameters are reasonably close to the arithmetic mean ± standard deviation of the individual Vd and K at 2.795 ± 1.597 L and 0.504 ± 0.322 h^{-1}. (Table 2). These facts suggest a proper simultaneous population modeling of levofloxacin plasma concentrations data.

CONCLUSION

Levofloxacin plasma concentration-time profiles in rabbits delivered by intravenous bolus injection and peroral administration can be described adequately by the population model using a two-compartment open model. The estimated disposition parameter models in each rabbit are different depending on the route of drug administration. Simultaneous fitting demonstrates the adequacy of fitting in all subjects.

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