Does the Anesthetic Urethane Influence the Pharmacokinetics of Antifungal Drugs? A Population Pharmacokinetic Investigation in Rats

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ABSTRACT: The aim of this paper was to analyze the impact of anesthesia induced by urethane on pharmacokinetics (PK) parameters of fluconazole (FCZ), mostly eliminated via renal excretion and voriconazole (VRC), eliminated mainly by hepatic metabolism. FCZ and VRC PK were investigated after administration of 10 mg/kg i.v. and 5 mg/kg i.v. doses to awake and urethane anesthetized Wistar rats ($n = 6$ per group), respectively. After dosing, blood samples were collected up to 18 h (FCZ) or 12 h (VRC) and the plasma data analysis was performed using the software MONOLIX v. 4.2.2. The population PK parameters and microconstants were determined by fitting plasma concentration–time profiles to two-compartment model for FCZ and three-compartment model for VRC. Fitting of FCZ plasma profiles after dosing to awake and anaesthetized animals resulted in a volume of distribution (V) of 9.3 and 8.1 L/kg, and k_{10} values of 0.12 and 0.14 h^{-1}, respectively. VRC plasma profiles in awake and anaesthetized showed V 8.7 of and 7.6 L/kg, and k_{10} of 0.15 and 0.16 h^{-1}, respectively. No statistical differences between plasma PK parameters and microconstants for the same drug in both animal conditions studied were observed ($\alpha = 0.05$). © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3314–3318, 2015
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Pharmacokinetic (PK) studies in awaken animals are difficult to perform because of their exposure to stress conditions. It is common to evaluate the PK properties of drug in anaesthetized laboratory animals, especially when it is necessary to do surgical procedures on them like in the microdialysis studies performed in our laboratory.[1–4] Even though anesthetized animals have been widely used in scientific studies, it is well known that anesthetic drugs could modify animal physiology introducing artifacts in pharmacological analysis, and these alterations depend on the drugs used and their dosage.[5] Among different anesthetics that are used in scientific studies, urethane is high employed mostly because of its long-lasting effect and less influence on autonomic, cardiovascular, and respiratory systems.[6] However, urethane may not be suitable for some studies because of its endocrine and renal effects.[7] Briefly, urethane owes its diuretic effect both to an increase of the glomerular filtration rate and a decrease of the tubular water reabsorption.[8] There are few studies regarding the urethane influence on PK properties of drugs. Two different researches have described that the use of urethane at high doses could affect drug metabolism through an inhibition of cytochrome CYP3A, changing PK properties of some drugs.[9,10] As urethane can be used alone or together with chloralose,[11] the mixture urethane–chloralose effect on the PK behavior of carvedilol in normotensive control rats and induced hypertensive animals was analyzed. The results showed that anesthesia did not modify the PK of carvedilol in both animal groups.[12]

Considering these observations, the aim of this work was to analyze the influence of the anesthetic drug urethane on the PK properties of two different antifungal drugs, fluconazole (FCZ) and voriconazole (VRC) after their intravenous administration to male Wistar rats. These drugs were selected as model drugs in this study as they are eliminated from the body through two different routes. FCZ is eliminated mainly by renal excretion[13] and VRC by hepatic metabolism through cytochrome P450.[14]

The PK study was carried out in male Wistar rats weighting approximately 250 g ($n = 6$ per group) and was approved by the Ethics committee of Universidade Federal do Rio Grande do Sul (Protocols #2008187 and #19544). FCZ and VRC were administered intravenously (i.v. bolus) through the tail vein at a dose of 10 and 5 mg/kg, respectively. On the day of the experiments, animals were anaesthetized with urethane (1.25 g/kg i.p.) and the carotid artery was used for blood collection in the anesthetized group. In the awaken animals group, the blood was collected from the lateral tail vein. Blood samples (200 μL) were collected into heparinized tubes at different time points (0, 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, and 18 h for FCZ and 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 12 h for VRC) from the tail vein and centrifuged at 11,952 g at 21°C for 10 min to separate the plasma. Plasma (100 μL) samples were prepared to quantify both drugs in plasma by liquid chromatography–mass spectrometry bioanalytical methods developed and validated according to US FDA guidelines,[15] as described previously.[3,16] Both methods were able to quantify the plasma samples as their calibration curves fitted the concentration range analyzed (10–2000 ng/mL for FCZ and 50–2500 ng/mL for VCR), with limits

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Table 1. Noncompartmental Pharmacokinetic (PK) Parameters of Fluconazole (FCZ) and Voriconazole (VRC) in Both Awake and Anaesthetized Wistar Rats ($n = 6$ per Group) After Single Intravenous Dose of 10 and 5 mg/kg, respectively

PK Parameter	VRC Awaken	VRC Anaesthetized	FCZ Awaken	FCZ[a] Anaesthetized
AUC (ng h/mL)	13,909 ± 1928	12,390 ± 4960	41,586 ± 8380	66,210 ± 15,920
ke (h^{-1})	0.153 ± 0.02	0.191 ± 0.04	0.105 ± 0.01	0.104 ± 0.02
$t_{1/2}$ (h)	4.7 ± 0.7	3.6 ± 0.3	6.6 ± 0.3	6.8 ± 1.1
V_{dss} (L/kg)	9.7 ± 2.1	10.6 ± 2.5	10.1 ± 3.2	8.5 ± 2.9
CL (L/h kg-1)	0.37 ± 0.05	0.45 ± 0.14	1.71 ± 0.25	1.63 ± 0.41
MRT (h)	4.9 ± 1.0	3.8 ± 0.4	8.5 ± 0.7	8.9 ± 0.5

[a]Data previously published by Azeredo et al.[18]
Mean ± SD.

Table 2. Population Pharmacokinetic (PK) Parameters of Fluconazole (FCZ) and Voriconazole (VRC) in Both Awake and Anaesthetized Wistar Rats ($n = 6$ per Group) After Single Intravenous Dose of 10 and 5 mg/kg, respectively

	Estimate (%RSE)				BSV (%RSE)			
	Awaken		Anesthetized		Awaken		Anesthetized	
PK Constant	FCZ	VRC	FCZ	VRC	FCZ	VRC	FCZ	VRC
V (L/kg)	9.3 (12)	8.7 (7)	8.1 (23)	7.6 (8)	1.8 (19)	1.6 (30)	1.3 (22)	1.9 (29)
k_{10} (h^{-1})	0.12 (21)	0.15 (9)	0.14 (16)	0.16 (8)	0.16 (14)	0.2 (17)	0.19 (27)	0.19 (24)
k_{12} (h^{-1})	2.43 (22)	3.21 (19)	2.81 (13)	3.09 (21)	0.16 (12)	0.27 (21)	0.21 (20)	0.31 (22)
k_{21} (h^{-1})	1.08 (17)	2.58 (26)	1.37 (23)	2.67 (18)	0.23 (21)	0.19 (24)	0.17 (16)	0.17 (15)
k_{13} (h^{-1})	–	1.5 (13)	–	1.9 (12)	–	0.49 (34)	–	0.24 (20)
k_{31} (h^{-1})	–	1.1 (26)	–	1.7 (15)	–	0.18 (27)	–	0.82 (31)

%RSE, percent relative standard error; BSV, between-subject variability (η).

of quantification of 10 and 50 ng/mL for FCZ and VRC, respectively. The samples with higher concentration values than the last point of the calibration curves were diluted in order to be properly quantified.

Data were analyzed using the nonlinear mixed-effect modeling software program Monolix® version 4.2.2 (http://www.lixoft.eu). Noncompartmental analysis was conducted using the software Excel® version 14.07140.5002. The area under the curve ($AUC_{0-\infty}$) was determined using the linear trapezoidal rule, the elimination rate constant (k_e) was determined by the slope of the plot of the logarithm of concentration against time, and half-life for the terminal slope ($t_{1/2}$) was calculated by the equation $0.693/k_e$. V_{dss}, CL, and Mean Residence Time (MRT) were calculated using the classic PK equations.[17,18] The PK parameters values are disposed in Table 1. It is important to highlight the statistically insignificant difference among these parameters when comparing the same drug administered to both awake and anaesthetized animal groups. For the population analysis, parameters and microconstants were estimated by computing the maximum likelihood estimation of the parameters without any approximation of the model (no linearization) using the stochastic approximation expectation maximization algorithm combined with a Markov Chain Monte Carlo procedure. A constant error model was used to describe the residual variability (ε_{CONST}), and the between-subject variability (BSV or η) was ascribed to an exponential error model. Parameter shrinkage was calculated as [1−SD(η)/ω], where SD(η) and ω are the standard deviation of individual η parameters and the population model estimate of the BSV, respectively. The likelihood ratio test including the log-likelihood, the Akaike information criterion and the Bayesian information criterion was used to test different hypotheses regarding the final model and residual variability model. From the final model, 1000 simulations were performed to compute the visual predictive check and the normalized prediction distribution error metrics, whose mean, variance, and distribution must not be different from 0, 1, and a normal distribution.[19,20]

A two-compartment open model was fitted to the FCZ plasma concentration in both awake and anaesthetized Wistar rats:

$$\frac{dC}{dt} = -k_{10}C - k_{12}C + k_{21}P$$

$$\frac{dP}{dt} = -k_{21}P + k_{12}C$$

where C and P are the amounts of drug in the central and peripheral compartments, respectively; k_{10} is the elimination rate from central compartment; k_{12} and k_{21} are the distribution rates from central to peripheral compartment and vice versa.

A three-compartment open model was fitted to the VRC plasma concentration in both awake and anaesthetized Wistar rats:

$$\frac{dC}{dt} = -k_{10}C - k_{12}C - k_{13}C + k_{21}P_s + k_{31}P_d$$

$$\frac{dP_s}{dt} = -k_{21}P_s + k_{12}C$$

$$\frac{dP_d}{dt} = -k_{31}P_d + k_{13}C$$

where C, P_s, and P_d are the amounts of drug in the central, shallow peripheral, and deep peripheral compartments, respectively; k_{10} is the elimination rate from central compartment;