

Pharmacokinetic Plasma Profile and Bioavailability Evaluation of Gatifloxacin in Rats

Leandro TASSO *¹, Clarissa C. BETTONI ¹ & Teresa DALLA COSTA ^{1,2}

¹ Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia,
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

² Centro Bioanalítico de Medicamentos, Faculdade de Farmácia,
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

SUMMARY. Although gatifloxacin has been clinically used for some years, data from pre-clinical studies are rare in the literature. The objective of this work was to determine the pharmacokinetics plasma parameters of gatifloxacin in rats after intravenous (6 mg/kg) and oral (12 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 (0.9 ± 0.2 and 1.0 ± 0.3 L/h/kg), the terminal half-life (3.3 ± 0.8 and 3.7 ± 0.3 h) and the apparent volume of distribution (2.8 ± 0.4 and 3.1 ± 1.0 L/kg) were statistically similar after i.v. and oral administration, by both model independent and compartmental approaches. The area under the curve was reduced after oral dosing (4.1 ± 1.6 $\mu\text{g}\cdot\text{h/mL}$) in comparison to i.v. dosing (6.6 ± 1.3 $\mu\text{g}\cdot\text{h/mL}$) leading to an oral bioavailability of 31%. The absorption was fast, with a constant rate of 5.0 ± 1.8 h⁻¹. The results evidenced the linear pharmacokinetics of gatifloxacin in rodents in the dose range of 6 to 12 mg/kg.

RESUMEN. "Perfil Farmacocinético Plasmático y Biodisponibilidad de Gatifloxacin en Ratas". Aunque la gatifloxacin ha sido usada en la clínica por algunos años, no son frecuentes los estudios pre-clínicos de este antibiótico. El objetivo de este trabajo fue determinar los parámetros farmacocinéticos en plasma de la gatifloxacin en ratas después de la administración intravenosa (6 mg/kg) y oral (12 mg/kg). Los datos experimentales fueron dispuestos adecuadamente en un modelo de dos-compartmentos después de la administración intravenosa y en un modelo de un compartimiento con la absorción de primer orden después de la administración oral. El clearance total ($0,9 \pm 0,2$ y $1,0 \pm 0,3$ l/h /kg), el semivida de eliminación ($3,3 \pm 0,8$ y $3,7 \pm 0,3$ h) y el volumen de distribución ($2,8 \pm 0,4$ y $3,1 \pm 1,0$ L/kg) fueron estadísticamente similares después de la administración i.v. y de la oral, para los modos de determinación independiente y dependiente del modelo. El área bajo la curva fue reducida después de la administración oral ($4,1 \pm 1,6$ $\mu\text{g}\cdot\text{h/mL}$) con respecto a la administración i.v. ($6,6 \pm 1,3$ $\mu\text{g}\cdot\text{h/mL}$) llevando a una biodisponibilidad oral de los 31%. La absorción fue rápida, con un índice constante de $5,0 \pm 1,8$ h⁻¹. Los resultados evidenciaron la farmacocinética lineal del gatifloxacin en roedores en la gama de la dosis 6-12 mg/kg.

INTRODUCTION

Gatifloxacin 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 pharmacokinetic characteristics, and lower potential for drug resistance. Similar to several other fluoroquinolones, gatifloxacin has enhanced potency against Gram-positive cocci, including multiple-drug-resistant *Streptococcus pneumoniae* isolates ².

Although the pharmacokinetic of oral gati-

floxacin has been studied extensively in volunteers ³⁻¹⁶, 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. This investigational approach in animals, even when the drug has already been used in humans, can support dosing regimen

KEY WORDS: Bioavailability, Gatifloxacin, Pharmacokinetics, Rodents.

PALABRAS CLAVE: Biodisponibilidad, Farmacocinética, Gatifloxacin.

* Autor a quem correspondência deve ser enviada. E-mail: leandrotasso@hotmail.com

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 gatifloxacin in rats after intravenous and oral administration.

MATERIALS AND METHODS

Drugs, reagents and solvents

Gatifloxacin (AM-1155) was a gift from Bristol-Myers Squibb (São Paulo, Brazil). Norfloxacin (internal standard) was obtained from Delaware® (Porto Alegre, Brazil). Urethane was purchased from Sigma (St. Louis, USA). Methanol and acetonitrile, HPLC grade, as well as triethylamine and phosphoric acid were purchased from Merck® (Porto Alegre, Brazil). Water was purified by a Milli-Q system (Millipore®). All other chemicals and solvents were of analytical grade.

Study protocol

The study was approved by the Ethics in Research Committee of the Federal University of Rio Grande do Sul (Protocol # 2005413). Male Wistar rats (250-300 g), purchased from Fundação Estadual para a Produção e Pesquisa em Saúde (FEPPS, Porto Alegre, Brazil) were used in the experiments. The rats were housed under standard conditions with room temperature of 21 ± 2 °C, humidity of approximately 65% and a 12-h light:12-h dark cycle. Water was freely available. Animals that received the dose by oral route were deprived of food for 12 h before experimentation.

The pharmacokinetic evaluation of gatifloxacin was conducted using two groups of animals. One group (n = 8) received a single intravenous bolus dose of gatifloxacin (6 mg/kg) injected into the lateral tail vein. The second group received a single dose of 12 mg/kg of gatifloxacin by oral gavage (n = 6). The doses were chosen based on human therapy. Gatifloxacin solution for intravenous (i.v.) and oral administration was prepared in 0.9% NaCl solution.

For plasma sampling rats were anesthetized with urethane (1.25 g/kg, i.p.) and a cannula was inserted into the carotid artery for blood sampling. At predetermined time 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, 21 ± 1 °C for 15 min and stored at -20 °C until analysis. A previously validated SPE-HPLC method was used to quantify the drug in the samples¹⁷.

Pharmacokinetic and statistical analysis

The individual gatifloxacin plasma profiles were analyzed by non-compartmental approach using traditional equations¹⁸. In addition, compartmental individual analysis of the plasma curves were conducted using the computer program Scientist® for Windows™ v. 2.1 (Micro-math®, Missouri, USA). A weighting factor of 1 (1/y) was used for fit all data sets. The goodness of fit of the chosen compartmental model was determined by the Model Selection Criterion (MSC) as well as by the correlation coefficient calculated by the program. The pharmacokinetic parameters determined by non-compartmental and compartmental analysis for both doses and administration routes investigated were compared by Student "t" test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The mean pharmacokinetic plasma profiles of gatifloxacin after i.v. and oral administration of 6 mg/kg and 12 mg/kg dose, respectively, are shown in Fig. 1. The experimental data were adequately fitted to a two-compartment model after intravenous dosing resulting in MSC values ranging from 3.10 to 4.39 and correlation coefficients from 0.988 to 0.997, showing a good agreement between the experimental data and the model selected. Regarding oral administration, a one-compartment model with first order absorption was more appropriate to describe the data, with MSC values ranging from 1.39 to 3.04 and correlation coefficients from 0.921 to 0.990.

A two-compartment model was also reported previously to describe gatifloxacin plasma profile after i.v. administration to normal rats¹⁹ when doses ranging from 25 to 100 mg/kg were employed, 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 1.

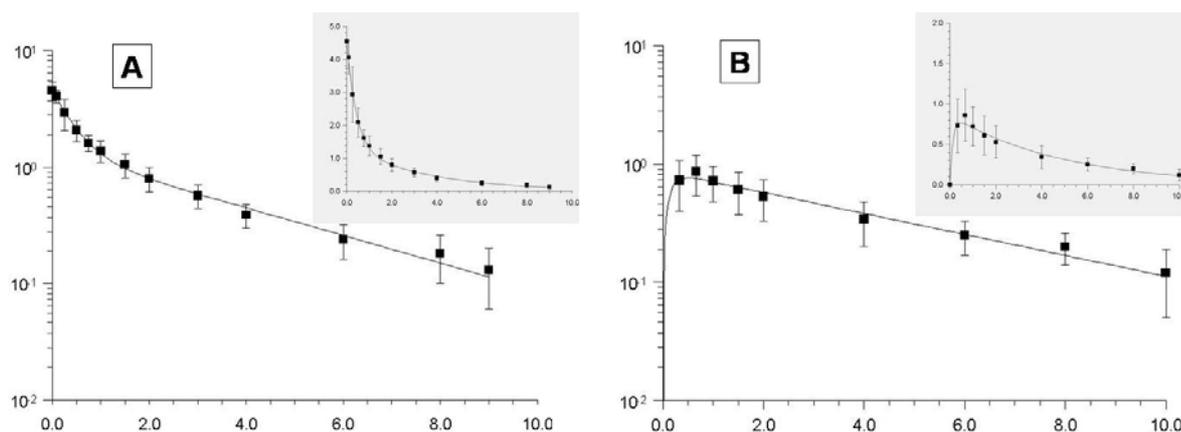


Figure 1. Mean plasma concentration-time profiles after gatifloxacin i.v. administration of 6 mg/kg (n = 8) (A) and oral administration of 12 mg/kg (n = 6) (B) to Wistar rats. In the details the plots in linear scale (mean \pm SD).

Parameters	6 mg/kg i.v. dosing (n = 8)		12 mg/kg oral dosing (n = 6)	
	Model independent	Two-compartment	Model independent	One-compartment
A ($\mu\text{g/mL}$)	-	3.3 ± 0.9	-	-
B ($\mu\text{g/mL}$)	-	1.3 ± 0.5	-	-
α (h^{-1})	-	2.7 ± 1.7	-	-
β or k_e (h^{-1})**	0.22 ± 0.05	0.28 ± 0.1	0.19 ± 0.01	0.22 ± 0.04
k_a (h^{-1})	-	-	-	5.0 ± 1.8
$t^{1/2}_{\text{elim}}$ (h)	3.3 ± 0.8	2.8 ± 0.9	3.7 ± 0.3	3.2 ± 0.6
$\text{AUC}_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$)	6.6 ± 1.3	6.3 ± 1.2	$4.1 \pm 1.6^*$	$3.7 \pm 1.2^*$
C_{max} ($\mu\text{g/mL}$)	-	-	0.86 ± 0.32	0.70 ± 0.19
T_{max} (h)	-	-	0.55 ± 0.27	0.71 ± 0.24
CL_{tot} (L/h/kg)	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	1.1 ± 0.3
V_c (L/kg)	-	1.4 ± 0.3	-	-
$V_{d_{\text{ss}}}$ (L/kg)	2.8 ± 0.4	3.0 ± 0.6	3.1 ± 1.0	4.9 ± 1.7
MRT (h)	3.1 ± 0.9	-	6.5 ± 0.5	-
f (%)	-	-	31	-

Table 1. Gatifloxacin pharmacokinetic parameters after single intravenous and oral administration to Wistar rats. *Statistical difference in comparison to i.v. parameter ($p < 0.05$). ** β for two-compartment, k_e for model independent and one-compartment.

There was no statistical difference between the PK parameters determined by compartmental and non-compartmental approaches. In the same manner, the total clearance (CL_{tot}), the terminal half-life ($t^{1/2}$) and the apparent volume of distribution ($V_{d_{\text{ss}}}$) 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 3.3 ± 0.8 h and 3.7 ± 0.3 h, respectively, different from that observed for health humans subjects, which was of 7.4 ± 1.6 h²⁰, demonstrating that gatifloxacin concentrations decline faster in rats than in humans. The area under the curve was reduced when the oral route was used, from 6.6 ± 1.3

$\mu\text{g}\cdot\text{h/mL}$ after i.v. dosing (6 mg/kg) to 4.1 ± 1.6 $\mu\text{g}\cdot\text{h/mL}$ after oral administration (12 mg/kg), leading to an oral bioavailability of 31%. We have previously reported the pharmacokinetics of gatifloxacin in rat after 6 mg/kg oral administration, resulting in an area under the curve (AUC) of 1.9 ± 0.4 $\mu\text{g}\cdot\text{h/mL}$ ¹⁷ which would result in similar bioavailability (29%). The oral absolute bioavailability in human is estimated in 96%¹⁰ which allows for comparable plasma concentrations at the same recommended dosage by either i.v. and oral routes. The data obtained showed that this condition does not hold true for rodents.

The absorption phase was rapid after oral

dosing resulting in an absorption rate constant (k_a) of $5.0 \pm 1.8 \text{ h}^{-1}$, which was higher than $1.61 \pm 0.8 \text{ h}^{-1}$ observed for the 6 mg/kg previously reported¹⁷. As the peak plasma concentration (C_{\max}) took place approximately 30 min after the 12 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 6 mg/kg oral dose was previously evaluated, the peak plasma level took place 90 min after dosing, allowing a better determination of the absorption rate constant.

It is known that the use of anesthetics can alter the pharmacokinetic disposition of other drugs given concomitantly directly affecting parameters such as clearance and volume of distribution and indirectly the other dependent parameters. The use of urethane in the present study, however, did not influence gatifloxacin disposition. When the present results are compared with those reported by Yshiwata *et al.* (2006) after 50 mg/kg i.v. dosing to awake animals¹⁹ the volume of distribution of the central compartment (V_c) and the total clearance (CL_{tot}) reported, $1.4 \pm 0.2 \text{ L/kg}$ and $1.0 \pm 0.1 \text{ L/h/kg}$, respectively, are similar to the parameters determined in the present study for the 6 mg/kg i.v. administration were a V_c of $1.4 \pm 0.3 \text{ L/kg}$ and a CL_{tot} of $1.0 \pm 0.2 \text{ L/h/kg}$ were obtained (Table 1). This work showed the absence of anesthetic influence on gatifloxacin pharmacokinetics.

CONCLUSIONS

The comparison of gatifloxacin pharmacokinetic parameters reported in the present paper and those previously reported in the literature^{17,19} allow the conclusion that the drug presents linear pharmacokinetics in rodents in the dose range from 6 to 12 mg/kg. Besides the oral bioavailability of gatifloxacin in rodents be approximately 1/3 of that reported for humans its faster elimination in rats lead to a smaller total drug exposition which probably will influence the bactericidal activity of this concentration dependent antimicrobial agent in animals. In this way, the conduction of experiments *in vivo* viewing to establish a PK/PD model for gatifloxacin in animals will have to take these differences into consideration.

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