Use of quantitative LC-MS/MS methods to compare conventional blood collection and microsampling in non-human primate

R. Cardin, S. Bertani, D. Federico, A. Donadi, F. Vinco, M. Pecoraro, A. Greco, and M. Breda
Aptuit, an Evotec Company, Via A. Fleming 4, 37135 Verona, Italy

Introduction

To obtain plasma, blood is generally withdrawn by a conventional venous collection method. Microsampling is a less invasive sampling technique (typically volume < 50 µL), which allows to reduce the stress correlated to the conventional blood sampling and to decrease the number of animals rodents for a preclinical study. The implementation of microsampling in non-human primate can reduce the stress and promote a positive interaction with technical staff which improves the overall well-being of the animal (refinement).

Objectives

The aim of this study was to compare PK parameters of selected drugs obtained after blood collection using the traditional method from femoral vein and microsampling from tail vein in Cynomolgous monkeys. Four drugs (Atorvastatin, Chloroquine, Naproxen and Sotalol) were selected based on acid-base and volume of distribution (Vd) properties.

Drug Characteristics

	MW	logP	pKa	Vd (L/Kg)	PPB (%)
Atorvastatin	558.6398	6.36	4.46	6	98
Naproxen	319.872	4.63	4.15	0.16	>99
Chloroquine	230.2592	3.29	10.1	200-800	46-74
Sotalol	272.364	0.85	10.07	1.2-2.4	0

Values sourced from DugBank.com

Sample Preparation

Protein precipitation was conducted with 7 µL of plasma samples (5 µL for Sotalol), spiked with internal standard (100 µL in acetonitrile for atorvastatin, 35 µL in methanol for Chloroquine, 200 µL in acetonitrile for Naproxen and Sotalol). After vortex mix, for atorvastatin the samples were centrifuged at 4000g for 10 min and 2 µL injected, for Chloroquine 60 µL of 0.01% in water were added and after centrifugation at 4000g for 10 min, 1.5 µL were injected; for Naproxen 200 µL of water were added and after centrifugation at 4000g for 10 min an aliquot 50 µL of the supernatant was further diluted with 150 µL of water and 2 µL injected; for Sotalol after the centrifugation 10 min at 4000g 20 µL of the supernatant were diluted with 500 µL of water/acetonitrile (20:80, v/v) containing 0.1% of formic acid and 2 µL were injected.

Method qualification

The plasma concentration of the 4 drugs were determined using 4 qualified methods. The qualification consisted of the evaluation of linearity, intra-run precision and accuracy, selectivity, carry-over and stability (4 h at room temperature and 3F/T cycles). The acceptance criteria were based on the international guideline [1, 2]. The methods were qualified in the calibration range of 0.25-250 for Atorvastatin, 0.5-500 ng/mL for Chloroquine, 50-50000 ng/mL for Naproxen and Sotalol.

Methods: LC and MS/MS conditions

	Atorvastatin	Naproxen	Chloroquine	Sotalol
Chromatography System	Waters Acquity UPLC	Waters Acquity UPLC	Waters Acquity UPLC	Waters Acquity UPLC
Analytical Column	Acquity UPLC BEH C18 Column, 130 Å, 1.7 µm, 2.1 x 50 mm, Waters	Acquity UPLC HSS T3 Column, 100 Å, 1.8 µm, 2.1 x 50 mm, Waters	Acquity UPLC HSS T3 Column, 100 Å, 1.8 µm, 2.1 x 50 mm, Waters	50 x 2.1mm i.d. Atlantis HILIC Type 3 µm, Waters
Mobile Phase A	5 mM Ammonium acetate	Water containing 0.1% (v/v)of formic acid	HFBA Buffer	10 mM Ammonium formate containing 0.02% (v/v)of formic acid
Mobile Phase B	Acetonitrile	Acetonitrile containing 0.1% (v/v)of formic acid	Acetonitrile	Acetonitrile containing 0.1% (v/v) of formic acid
Flow Rate	0.6 mL/min	0.6 mL/min	0.6 mL/min	0.5 mL/min
Mass Spectrometer	Waters Xevo TQS	Waters Xevo TQS	Waters Xevo TQS	Applied Biosystems/MDS Sciex API-4000
Ionisation Interface	ZSpray™	Zspray™	Zspray™	TurboIonSpray™
Chromatrographic conditions	Isocratic	Isocratic	Gradient	Isocratic

	Q1 (m/z)	Q1 (m/z)	TEM (°C)	CE (eV)
Atorvastatin	322	249	350	16
$[^2H_5]$-Atorvastatin	564	445	350	16
Chloroquine	322	249	500	20
$[^2H_{10}]$-Chloroquine	330	247	500	20
Naproxen	231	185	450	12
$[^2H_3]$-Naproxen	234	188	450	12
Sotalol	273	213	600	22
$[^2H_7]$-Sotalol	280	214	600	21

PK and statistical analysis

The pharmacokinetic parameters were calculated with non-compartmental model using WinNonlin version software. Values for C_{max} and AUC0-tz were determined and expressed as mean ± standard deviation (SD). T_{max} evaluation and expressed as median.

Drugs	Blood source	T_{max} (h)	C_{max} (ng/mL)	AUC0-tz# (h*ng/mL)
Atorvastatin	Standard Femoral vein	1	47.7 ± 36.9	164 ± 110
	Microsampling Tail vein	1	44.7 ± 29.3	203 ± 150
Naproxen	Standard Femoral vein	1	216 ± 56.6	2625 ± 661
	Microsampling Tail vein	1	524 ± 234	3415 ± 1054
Chloroquine	Standard Femoral vein	2	71300 ± 8750	290000 ± 40342
	Microsampling Tail vein	2	69067 ± 12855	289000 ± 43734
Sotalol	Standard Femoral vein	1	4720 ± 1020	31067 ± 3739
	Microsampling Tail vein	1	3803 ± 671	31950 ± 5277

Pharmacokinetic Parameters in plasma obtained from blood collected using standard procedure from femoral vein and microsampling from tail vein

Conclusions

Significant differences in the pharmacokinetic parameters were observed only for chloroquine in response to the different sampling methods. After microsampling collection Chloroquine C_{max} and AUC were 2.4 and 1.3 higher, respectively than that after standard collection from femoral vein. For Sotalol, even if the C_{max} was 0.8 lower with microsampling, the AUC values were comparable. Atorvastatin AUC after microsampling collection was 1.2 higher while no differences were observed for C_{max}. No influence from the different sampling methods were observed on PK parameters of Naproxen. No differences on T_{max} were observed for all tested drugs.

References

1. FDA, Guidance for Industry: Bioanalytical Method Validation. (2018).
2. EMEA, Guideline on bioanalytical method validation. (2011).