

Comparison of Serial and Parallel Blood Sampling Techniques in Mouse Pharmacokinetics Study

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ABSTRACT

Obtaining early stage pharmacokinetics (PK) data in the evaluation of new chemical entities is a prerequisite for successful animal pharmacology and toxicology studies. Quantitative measures of drug exposure are key components needed for the sound interpretation of preclinical efficacy studies. In early drug discovery stage, animal PK studies are often performed in mice, which are one of the most common animal models used to assess preclinical efficacy. Because

of the small body size of mouse, parallel blood sampling is generally used, i.e. each mouse is subject to only one blood draw through cardiac puncture. Thus, for a one-route, 7-time point PK study, a minimum of 21 mice would be needed. To reduce the number of animals used and, furthermore, to reduce the amount of test material needed, we evaluated serial blood sampling technique in mouse PK studies. Specifically, each mouse was administered with antipyrine, caffeine, or erythromycin via tail vein at 5 mg/kg. Blood samples were collected at 5, 15, 30, 60, 180, 360, and 1440 min from each animal via saphenous vein, with 20-30 μ L withdrawn at each time point. Fifteen microliters of blood sample were then submitted for quantitative bioanalysis by HPLC-MS/MS. The results showed that the blood concentrations of antipyrine and erythromycin at later time points were higher using serial sampling technique than those from parallel sampling technique, while blood concentration levels for caffeine were similar for both serial and parallel sampling. The PK parameters calculated using non-compartmental analysis (NCA) indicated that clearance values (Cl) for antipyrine and erythromycin obtained from either technique were similar, while volumes of distribution (Vd) obtained from serial sampling were greater than those from parallel sampling, suggesting an apparent increase in distribution to tissues. Both Cl and Vd obtained for caffeine were similar whether a serial or parallel sampling technique was used. The differences of caffeine from the other two compounds were that the former had lower Cl and displayed monoexponential elimination kinetics, while the latter two had higher Cl and displayed multiexponential elimination kinetics. Our study shows that serial and parallel sampling techniques may generate similar PK parameters for some compounds, but for other compounds the results may be different. More studies are needed to elucidate the discrepancies obtained using these two sampling techniques.

INTRODUCTION

1. Mice are one of the most used animal models for preclinical efficacy and PK assessment in early drug discovery stage,
2. Because of the small body size of mice, parallel blood sampling is generally used, i.e. each mouse is subject to only one blood draw through cardiac puncture.
3. One issue with parallel sampling is that the number of animals required can be large, depending on the number of time points and replications. Consequently, a large amount of test compound is needed for administration. At discovery stage, there are many tests needed to be done while the amount of compound is often limited.
4. Another disadvantage is that PK parameters obtained from the parallel sampling can not evaluate individual difference among animals.
5. To address the above mentioned issues, we have developed serial blood sampling technique in mouse PK studies, i.e. the blood samples for the whole time course are collected from a single mouse.

MATERIALS AND METHODS

ANIMALS, CHEMICALS AND MATERIALS

Male CD-1 mice [CrI:CD1(ICR)], weighing 20-30 g, were purchased from Charles River Laboratories (Wilmington, MA - USA). All test compounds were purchased from Sigma (St. Louis, MO - USA). Microvette Lithium-Heparin coated capillary tubes and vials were purchased from Sarstedt (Germany).

ANIMAL WORK AND ANALYSIS

1. Each mouse was administered with test compound via tail vein at 5 mg/kg (dosing volume 5 mL/kg).
2. Total of 3 animals were used for each independent serial sampling experiment. Blood samples from whole time course (5, 15, 30, 60, 180, 360, and 1440 min) were collected from each animal via saphenous vein; 20-30 μ L withdrawn at each time point.
3. Total of 21 animals were used for each independent parallel sampling experiment. Blood sample at each time point (5, 15, 30, 60, 180, 360, or 1440 min) was collected from one animal via cardiac puncture (typically 300 μ L is collected).
4. Fifteen microliters of blood sample were then submitted for quantitative bioanalysis by HPLC-MS/MS.
5. The fundamental pharmacokinetic parameters (half-life, clearance, volume of distribution and AUC) were obtained from the non-compartmental analysis using WinNonlin.

REFERENCE

Diehl et al. (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol.* 21: 15-23.

RESULT

1. The validation results obtained from serial sampling were compared to those from parallel sampling (besides the original 3 compounds mentioned in Abstract, 2 more compounds were added).
2. Blood drug concentration-time profiles and PK parameters are presented above.
3. Overall the parameters generated from these two sampling methods are relatively close, especially those for caffeine, which showed a low Cl.

PHARMACOKINETIC PARAMETERS & CONCENTRATION-TIME PROFILES

AFTER IV ADMINISTRATION TO MICE WITH ANTIPYRINE (5 MG/KG)

SAMPLING	PARALLEL	SERIAL	CONCENTRATION-TIME PROFILE
T1/2 (min)	34 \pm 7	48 \pm 12	
Cl (mL/min/kg)	91 \pm 14	92 \pm 19	
Vz (mL/kg)	4459 \pm 1595	6360 \pm 1904	
Vss (mL/kg)	3485 \pm 1173	5235 \pm 1162	
AUClast (min*ng/mL)	55168 \pm 8277	55183 \pm 11105	
AUCINF (min*ng/mL)	55771 \pm 8578	56705 \pm 12931	

Results are mean \pm SE, n=3 independent experiments

AFTER IV ADMINISTRATION TO MICE WITH CAFFEINE (5 MG/KG)

SAMPLING	PARALLEL	SERIAL	CONCENTRATION-TIME PROFILE
T1/2 (min)	47 \pm 17	47 \pm 17	
Cl (mL/min/kg)	20 \pm 5	21 \pm 6	
Vz (mL/kg)	1335 \pm 502	1385 \pm 504	
Vss (mL/kg)	1316 \pm 461	1376 \pm 443	
AUClast (min*ng/mL)	238283 \pm 54051	231819 \pm 59935	
AUCINF (min*ng/mL)	259550 \pm 64197	251969 \pm 67512	

Results are mean \pm SE, n=3 independent experiments

AFTER IV ADMINISTRATION TO MICE WITH CIPROFLOXACIN (5 MG/KG)

SAMPLING	PARALLEL	SERIAL	CONCENTRATION-TIME PROFILE
T1/2 (min)	317 \pm 23	448 \pm 107	
Cl (mL/min/kg)	66 \pm 14	80 \pm 52	
Vz (mL/kg)	30281 \pm 8144	49180 \pm 28064	
Vss (mL/kg)	29599 \pm 14360	59376 \pm 41201	
AUClast (min*ng/mL)	71909 \pm 17226	69245 \pm 40456	
AUCINF (min*ng/mL)	78398 \pm 17536	87316 \pm 46835	

Results are mean \pm SE, n=3 independent experiments

AFTER IV ADMINISTRATION TO MICE WITH DEXTROMETORPHAN (5 MG/KG)

SAMPLING	PARALLEL	SERIAL	CONCENTRATION-TIME PROFILE
T1/2 (min)	54 \pm 26	45 \pm 10	
Cl (mL/min/kg)	169 \pm 6	131 \pm 43	
Vz (mL/kg)	13191 \pm 6054	8293 \pm 2642	
Vss (mL/kg)	11010 \pm 4874	7160 \pm 1555	
AUClast (min*ng/mL)	26779 \pm 1996	41856 \pm 16597	
AUCINF (min*ng/mL)	29602 \pm 1040	42591 \pm 16624	

Results are mean \pm SE, n=3 independent experiments

AFTER IV ADMINISTRATION TO MICE WITH ERYTHROMYCIN (5 MG/KG)

SAMPLING	PARALLEL	SERIAL	CONCENTRATION-TIME PROFILE
T1/2 (min)	28	41 \pm 1	
Cl (mL/min/kg)	102	108 \pm 25	
Vz (mL/kg)	4058	6288 \pm 1411	
Vss (mL/kg)	2839	4848 \pm 1073	
AUClast (min*ng/mL)	48252	52466 \pm 16922	
AUCINF (min*ng/mL)	48937	53976 \pm 16316	

Results are from 1 experiment (mean \pm SE, n=3 subjects, if applicable)

DISCUSSION

1. We have established a technique for serial sampling from one mouse, which generated the same quality of data as traditional parallel sampling.
2. The average circulating blood volume for mice is 72 mL/kg (Diehl et al, 2001). For a 25 mg mouse, the blood volume would be 1.8 mL. The total blood volume we sampled from one mouse is around 175 μ L throughout the serial sampling study, about 10% of total circulating blood volume.
3. The animals did not show any observed adverse effect, suggesting the amount of blood loss is tolerable to the animals.
4. Although not significant, there are differences in some parameters generated from these two methods for some compounds. Further studies are needed to understand the factors that caused those differences.
5. Each sampling method has its own advantages and disadvantages (see table below). If the number of animals and the amount of test compound are an issue, the serial sampling method provides an alternative way to conduct mouse PK at early drug discovery stage.

	ADVANTAGES	DISADVANTAGES
SERIAL SAMPLING	<ul style="list-style-type: none"> Requires fewer animals Requires less test compound Able to compare individual difference 	<ul style="list-style-type: none"> Complicated technique Small sample size Unable to harvest plasma Potential excessive blood loss
PARALLEL SAMPLING	<ul style="list-style-type: none"> Simple technique Large sample size Able to harvest plasma 	<ul style="list-style-type: none"> Requires more animals Requires more test compound Unable to compare individual difference