

# Study of Mouse Pharmacokinetics Using Serial Blood Sampling Technique

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## ABSTRACT

Mice are one of the most common animal models used in drug discovery stage.

Because of their small body size, parallel blood sampling is generally used in mouse pharmacokinetic (PK) studies, i.e. each mouse is subject to only one blood draw through cardiac puncture. One issue with parallel sampling is that the number of animals required can be large, depending on the numbers of time points and replicates. Consequently, a large amount of test compound is needed for administration.

To address this issue, we evaluated serial blood sampling technique in mouse PK studies using 5 test compounds (antipyrine, caffeine, ciprofloxacin, dextromethorphan, and erythromycin). Each test compound was injected 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were collected at 5, 15, 30, 60, 180, 360, and 1440 min from each animal via saphenous vein, with 20-30  $\mu$ L withdrawn at each time point. Fifteen microliters of blood sample were then submitted for quantitative bioanalysis by HPLC-MS/MS. The PK parameters were calculated using non-compartmental analysis (NCA).

The results obtained using serial sampling technique were compared to those using parallel sampling technique. For caffeine, the parameters obtained using both techniques are almost the same; caffeine has the lowest clearance value (Cl) among those tested compounds. For most of other tested compounds, AUC and Cl are also close. Volume of the distribution (Vd) is the parameter which shows the greatest discrepancy between these two techniques for the majority of tested compounds. Animals may be under more stress using serial sampling and excessive loss of blood is also a potential issue.

Is the discrepancy in Vd related to stress, blood loss, or clearance characteristics of compound? More investigations are needed to answer these questions. In summary, our study demonstrates that serial sampling technique would generate results comparable to those using parallel sampling technique and, therefore, is a useful alternative method for mouse PK study.

## INTRODUCTION

- Mice are one of the most common animal models for preclinical efficacy and PK assessment in early drug discovery stage,
- 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.
- However, the parallel sampling could generate several issues. First, the number of animals used could be large, depending on the numbers of time points and replicates.
- Consequently, much more amount of test compound would be needed for dosing to so many animals. At discovery stage, there are many tests needed to be done while the amount of compound is often limited.
- Furthermore, PK parameters obtained from the parallel sampling could not evaluate individual difference among animals because the concentration-time profile is not generated from a single animal.
- 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 & METHODS

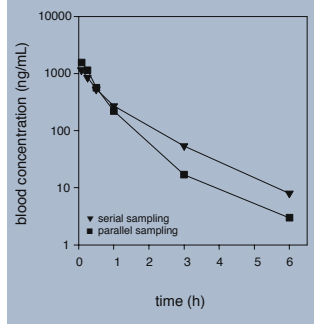
### ANIMALS, CHEMICALS AND MATERIALS

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

### ANIMAL WORK AND ANALYSIS

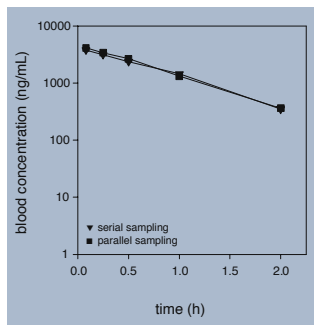
- Each mouse was administered with test compound via tail vein at 5 mg/kg (dosing volume 5 mL/kg).
- Total of 3 animals were used for each independent serial sampling experiment. Blood samples were collected at 5, 15, 30, 60, 120 (or 180), 360, and 1440 min from each animal via saphenous vein; 20-30  $\mu$ L withdrawn at each time point.
- Total of 21 animals were used for each independent parallel sampling experiment. Blood sample was collected at 5, 15, 30, 60, 120 (or 180), 360, or 1440 min from each animal via cardiac puncture (typically 300  $\mu$ L is collected, 3 mice were used at each time point for an independent experiment with total 7 time points).
- Fifteen microliters of blood sample were then used for quantitative bioanalysis by HPLC-MS/MS.
- The fundamental pharmacokinetic parameters (half-life, clearance, volume of distribution and AUC) were obtained from the non-compartmental analysis using WinNonlin.

## PK parameters for antipyrine (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	34 $\pm$ 7	48 $\pm$ 4	
Cl (mL/min/kg)	91 $\pm$ 8	92 $\pm$ 6	
Vz (mL/kg)	4459 $\pm$ 921	6360 $\pm$ 635	
Vss (mL/kg)	3485 $\pm$ 677	5235 $\pm$ 387	
AUClast (min*ng/mL)	55168 $\pm$ 4779	55183 $\pm$ 3702	
AUCINF (min*ng/mL)	55771 $\pm$ 4953	56705 $\pm$ 4310	

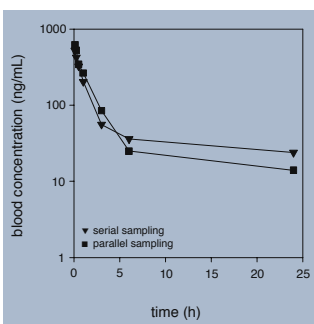
Results are mean  $\pm$  SE, from 3 independent experiments

## PK parameters for caffeine (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	32 $\pm$ 2	32 $\pm$ 2	
Cl (mL/min/kg)	23 $\pm$ 4	25 $\pm$ 2	
Vz (mL/kg)	1075 $\pm$ 186	1138 $\pm$ 116	
Vss (mL/kg)	1092 $\pm$ 172	1189 $\pm$ 116	
AUClast (min*ng/mL)	210218 $\pm$ 29722	200123 $\pm$ 15971	
AUCINF (min*ng/mL)	223145 $\pm$ 29702	212309 $\pm$ 16475	

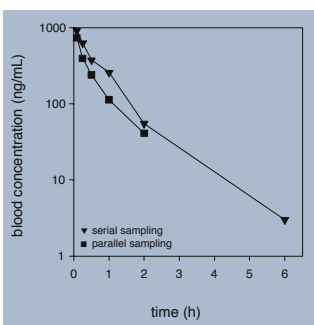
Results are mean  $\pm$  SE, from 3 independent experiments

## PK parameters for ciprofloxacin (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	545 $\pm$ 69	516 $\pm$ 43	
Cl (mL/min/kg)	64 $\pm$ 7	79 $\pm$ 17	
Vz (mL/kg)	50584 $\pm$ 9329	60478 $\pm$ 14198	
Vss (mL/kg)	39647 $\pm$ 12871	69735 $\pm$ 17865	
AUClast (min*ng/mL)	68044 $\pm$ 8942	67194 $\pm$ 13594	
AUCINF (min*ng/mL)	79785 $\pm$ 8805	87396 $\pm$ 15383	

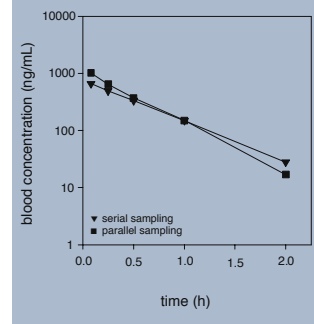
Results are mean  $\pm$  SE, from 3 independent experiments

## PK parameters for dextromethorphan (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	37 $\pm$ 5	43 $\pm$ 3	
Cl (mL/min/kg)	195 $\pm$ 12	142 $\pm$ 12	
Vz (mL/kg)	10506 $\pm$ 2021	8784 $\pm$ 953	
Vss (mL/kg)	8638 $\pm$ 1597	6752 $\pm$ 587	
AUClast (min*ng/mL)	24024 $\pm$ 2297	37251 $\pm$ 3959	
AUCINF (min*ng/mL)	25764 $\pm$ 1538	37786 $\pm$ 3949	

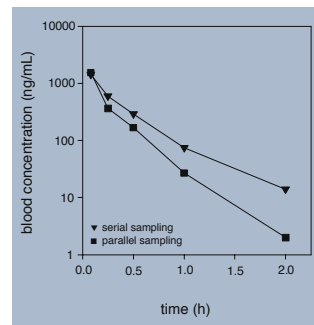
Results are mean  $\pm$  SE, from 3 independent experiments

## PK parameters for erythromycin (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	21 $\pm$ 2	25 $\pm$ 3	
Cl (mL/min/kg)	169 $\pm$ 37	194 $\pm$ 19	
Vz (mL/kg)	5339 $\pm$ 1771	6786 $\pm$ 947	
Vss (mL/kg)	4457 $\pm$ 736	7180 $\pm$ 985	
AUClast (min*ng/mL)	32416 $\pm$ 7575	26541 $\pm$ 2374	
AUCINF (min*ng/mL)	32941 $\pm$ 7605	27722 $\pm$ 2465	

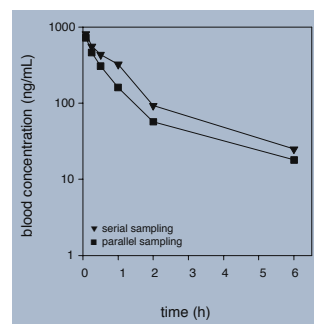
Results are mean  $\pm$  SE, from 3 independent experiments

## PK parameters for midazolam (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	13 $\pm$ 1	20 $\pm$ 1	
Cl (mL/min/kg)	191 $\pm$ 8	164 $\pm$ 11	
Vz (mL/kg)	3520 $\pm$ 529	4654 $\pm$ 316	
Vss (mL/kg)	2347 $\pm$ 321	3472 $\pm$ 302	
AUClast (min*ng/mL)	26104 $\pm$ 1048	31327 $\pm$ 2146	
AUCINF (min*ng/mL)	26205 $\pm$ 1106	31767 $\pm$ 2230	

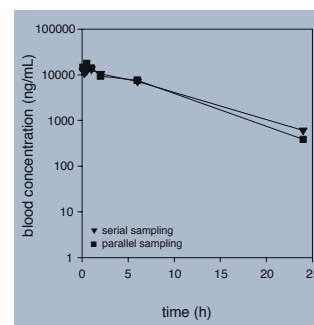
Results are mean  $\pm$  SE, from 3 independent experiments

## PK parameters for propranolol (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	100 $\pm$ 18	80 $\pm$ 7	
Cl (mL/min/kg)	162 $\pm$ 55	99 $\pm$ 16	
Vz (mL/kg)	21095 $\pm$ 4505	11713 $\pm$ 2249	
Vss (mL/kg)	13759 $\pm$ 2166	10845 $\pm$ 2295	
AUClast (min*ng/mL)	36462 $\pm$ 12070	60398 $\pm$ 11090	
AUCINF (min*ng/mL)	39434 $\pm$ 13568	63836 $\pm$ 12013	

Results are mean  $\pm$  SE, from 3 independent experiments

## PK parameters for sulfadiazine (5 mg/kg)

	Parallel sampling	Serial sampling	Concentration time profile
T1/2 (min)	270 $\pm$ 5	312 $\pm$ 20	
Cl (mL/min/kg)	0.79 $\pm$ 0.06	0.76 $\pm$ 0.03	
Vz (mL/kg)	308 $\pm$ 25	336 $\pm$ 14	
Vss (mL/kg)	318 $\pm$ 27	344 $\pm$ 12	
AUClast (min*ng/mL)	6251918 $\pm$ 467181	6387994 $\pm$ 234482	
AUCINF (min*ng/mL)	6401859 $\pm$ 467785	6687976 $\pm$ 296752	

Results are mean  $\pm$  SE, from 3 independent experiments

## RESULTS

- The results obtained from serial sampling were compared to those from parallel sampling (Besides original 5 compounds mentioned in Abstract, 3 more compound were added).
- Blood drug concentration-time profiles and PK parameters are presented.
- Overall the parameters generated from these two sampling methods are relatively close, especially those for caffeine and sulfadiazine, which showed low Cl.

## DISCUSSION

- We have established a technique for serial sampling using saphenous vein in mice.
- 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 approximately 175  $\mu$ L, representing about 10% of the total circulating blood volume.
- The animals did not show any observed adverse signs, suggesting the amount of blood loss is tolerable to the animals.
- There are differences in some parameters generated from serial and parallel sampling methods for some compounds. Interestingly, the two compounds, caffeine and sulfadiazine, that generated almost the same values in all parameters have low Cl. Further studies are needed to understand the factors that caused those differences.
- Each sampling method has its own advantages and disadvantages. If the number of animals and/or the amount of test compound are an issue, the serial sampling method provides an alternative way to conduct mouse PK studies at early drug discovery stage.

## COMPARISON OF SERIAL AND PARALLEL SAMPLING METHODS

	Advantages	Disadvantages
<b>SERIAL SAMPLING</b>	<ul style="list-style-type: none"> <li>Using fewer animals</li> <li>Using less amount of test compound</li> <li>Able to compare individual difference</li> </ul>	<ul style="list-style-type: none"> <li>Small sample size, which is only enough for one testing</li> <li>Potential stress on animals and excessive blood loss</li> </ul>
<b>PARALLEL SAMPLING</b>	<ul style="list-style-type: none"> <li>Large sample size, which can be used for retesting or other assays</li> </ul>	<ul style="list-style-type: none"> <li>Using more animals</li> <li>Using more test compound</li> <li>Unable to compare individual difference</li> </ul>

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.