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.

Materials and Methods

> ANIMALS, CHEMICALS AND MATERIALS

Mice: CD1 mice (CD:1(Sam), Harlan) weighing 20-30 g were purchased from Charles River Laboratories (Kingston, MA, USA).

All test compounds were purchased from Sigma (St. Louis, MO, USA). Microvettes 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. Blood samples were collected at 5, 15, 30, 60, 180, 360, and 1440 min from each animal via saphenous vein, with 20-30 µL withdrawn at each time point. Fifteen microliters of blood sample was then submitted for quantitative bioanalysis by HPLC-MS/MS.

2. Total of 3 animals were used for each independent serial sampling experiment. Blood samples at each time point (5, 15, 30, 60, 180, 360, or 1440 min) was collected from one animal via saphenous vein; 20-30 µL withdrawn at each time point. Sample at each time point (5, 15, 30, 60, 180, 360, or 1440 min) was collected from one animal via cardiac puncture (typically 300 µL is collected).

3. Total of 21 animals were administered with test compound via tail vein at 5 mg/kg. Blood samples were collected from whole time course (5, 15, 30, 60, 180, 360, and 1440 min) were collected from each animal via saphenous vein; 20-30 µL withdrawn at each time point.

> Results

Further studies are needed to elucidate the discrepancies observed using these two sampling techniques.

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 µL/kg (Diehl et al., 2001). For a 25-g mouse, the blood volume would be 1.8 mL. The total blood volume we sampled from one mouse is around 175 µL throughout the whole time course, which is a very small sample size.

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 these 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 approach to conduct mouse or rat PK in drug discovery.