Mouse Pharmacokinetic Study of Ceftriaxone Using Mitra™ Microsampling Devices
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Purpose
Mice are one of the most commonly used animals in drug discovery pharmacokinetic (PK) studies. Guidelines are established for the frequency and amount of blood that can be collected from a single animal. Since the mouse’s body size is limited, parallel blood sampling is generally used whereby multiple mice are subjected to a single blood draw through cardiac puncture. One issue with parallel sampling, however, is that the number of animals required for the study can be large, depending on the number of time points and sample replicates required. Consequently, a large amount of test compound is also needed for dose administration. Repeated or serial sampling in a single animal, on the other hand, is often difficult, especially when cannulation is not an option. MitraTM microsampling devices (Neoteryx LLC) offer an alternative method for serial blood sampling in mouse PK studies. Using serial blood sampling methods rather than parallel blood sampling methods may greatly reduce the number of animals needed and lead to more reliable data by excluding individual differences. In this study, an experiment was designed to compare the serial blood sampling method using the MitraTM microsampling device with the parallel blood sampling method.

Methods
Ceftriaxone was administrated intravenously (IV) at 1 mg/kg and orally (PO) at 5 mg/kg. Blood samples (10 μL) were collected from the lateral tail vein at 0.083, 0.25, 0.5, 1, 2, 4, 7, and 24 hours after the IV dose and at 0.25, 0.5, 1, 2, 4, 7, and 24 hours after the PO dose. The serial bleeding mouse blood samples (10 μL) were collected by using MitraTM microsampling device. The conventional mouse PK samples using a single venipuncture from individual animals were collected by both MitraTM microsampling devices (10 μL) and K2EDTA coated tubes simultaneously. Anticoagulant containing blood samples were centrifuged and 100 μL of plasma was placed into Eppendorf tubes. The plasma samples were stored at -20°C for LC-MS/MS bioanalysis use. The blood samples were collected using the two sampling techniques were measured using liquid chromatography–tandem mass spectrometry, and PK parameters were evaluated using noncompartmental analysis.

Results
Whole blood and plasma PK parameters were calculated using non-compartmental models by the Phoenix® WinNonlin® 6.3 version software package. Ceftriaxone had low systemic clearance of 2.312 mL/min/kg via the MITRATM microsampling serial bleeding technique compared to hepatic blood flow (Mouse HBF = 90 mL/min/kg) through IV administration and a low volume of distribution (0.270 L/kg) compared to the total body water (Mouse TBW = 0.725 L/kg). Ceftriaxone also had very similar low systemic clearance of 2.082 mL/min/kg via MITRATM microsampling with individual time point termination through IV administration and a low volume of distribution (0.279 L/kg) as well.

Blood concentrations after oral administration reached a mean Cmax value of 380 ng/mL at 1 hr. The oral half-life after PO administration was 5.70 hours which is longer than that after IV administration (3.82 hours). The oral bioavailability was calculated using AUCinf calculations. The compound showed low systemic exposure after oral administration (F = 5.3%). Ceftriaxone had very similar PK parameter via MITRA technique with individual time point termination through PO arm. The oral half-life was 6.39 hours which is longer than that after IV administration (3.29 hours). The oral bioavailability is 2.4%.

Conclusion
After IV and PO administration of ceftriaxone, the concentration results and PK parameters from using the serial blood sampling method were comparable to those obtained from using the parallel blood sampling method. MitraTM microsampling devices provide a methodology that can be used for serial blood sampling in drug discovery mouse PK studies, and which could reduce costs, improve animal welfare, and save precious test articles.