Application of Mitra[®] microsampling for pharmacokinetic bioanalysis of monoclonal antibodies in rats

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Aim: Volumetric absorptive microsampling (VAM) is being increasingly applied in nonclinical pharmacokinetic studies. Although there are published results for VAM use in small molecule pharmacokinetics (PK) studies, there is limited data on the utility of VAM for protein therapeutics. **Results:** We describe the use of Mitra[®] microsampler for blood sampling, ELISA quantitation and PK analysis of two marketed therapeutic monoclonal antibodies administered to rat. Results generated for these monoclonal antibodies using Mitra[®] were compared with both serum and whole blood sampling methods in the same study. **Conclusion:** The low relative standard deviation among the three sets of PK data suggest that Mitra[®] microsampler could be useful in early nonclinical PK studies for protein therapeutics where reduction and refinement of animal use is desirable.

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Rodent models are commonly used in preclinical pharmacokinetic/pharmacodynamic (PK/PD) evaluation of new biological entity (NBE) drugs. Because protein therapeutics typically displays longer half-lives than small molecule drugs, an extended sampling time course is required to accurately characterize the PK/PD of these molecules. However, Institutional Animal Care and Use Committee (IACUC) regulations strictly limit volume and frequency of blood sampling for preclinical studies in small animals, especially mice, making accurate PK characterization challenging. In order to comply with IACUC restrictions, dosing of additional animals and utilization of staggered sampling is frequently employed, neither of which is desirable due to the intensification of animal use and likely introduction of additional interanimal variability in such composite PK/PD data.

The growing number of protein therapeutics in drug discovery along with the increased sensitivity of bioanalytical technologies are driving the development of novel approaches for reductions in both sample volume and animal usage [1]. In the past decade, volumetric absorptive microsampling (VAM) innovations such as whole blood liquid and dried blood spot have been contributing to improvements in the three Rs: replacement, reduction and refinement of animal usage [2–4]. Although whole blood microsampling [5–7] allows for reduced sample size and animal use, refrigeration and freezing of samples over the study duration is required to ensure sample quality. On the other hand, while dried blood spot sampling techniques can eliminate the need for temperature controls [8], the sample processing and extraction procedures are cumbersome [9] and results may be impacted by hematocrit [10].

The FDA Class 1, CE/IVD Mitra[®] microsampler has been shown as a promising alternative to dried blood spots for less impact of hematocrit [11–15]. An accurate sampling of either 10 or 20 μ l whole blood (two different microsampler sizes) is absorbed onto the tip of the microsampler, which is then permitted to dry at ambient temperature prior to sample analysis. The amount of time and resources saved by applying Mitra[®] microsampler has been detailed in previous studies [16].

In this work, rat intravenous and subcutaneous PK studies of two marketed therapeutic monoclonal antibody (mAb) drugs, trastuzumab and daclizumab, were conducted. The two mAbs were immediately available in house with enough amounts for rat PK study when this research proposal was submitted. Trastuzumab, sold under the

brand name Herceptin[®], is a monoclonal antibody used to treat breast cancer that is HER2 receptor positive. daclizumab, sold under the brand name Zinbryta[®], is a therapeutic humanized monoclonal antibody used for the treatment of adults with relapsing forms of multiple sclerosis. Three different sampling methods (serum, liquid whole blood and Mitra[®] micro sampler) were applied. A simple protein drug extraction from the Mitra[®] microsampler was followed by bioanalytical assay for drug quantitation. Drug PK data from the three sets of samples were obtained and compared.

Materials & experimental methods

Reagents

Trastuzumab and daclizumab used in this work were obtained from BIPI Molecule Discovery Group in Biotherapeutics (CT, USA). The rat serum and fresh whole blood were purchased from Bioreclamation IVT (MD, USA). The blocking reagent used in the bioanalytical assay was 5% (w/v) bovine serum albumin (SeraCare Life Sciences, MA, USA) in PBS (Gibco Laboratories, MD, USA). The ELISA capture was goat anti-human IgG (Novus, CO, USA) and the detection was HRP-conjugated goat anti-human IgG (Southernbiotech, AL, USA). Total of 20 µl of Mitra[®] microsampler (Neoteryx, CA, USA) were used in this work.

Bioanalytical method

A generic anti-human IgG capture and HRP-conjugated anti-human IgG detection were paired in the ELISA method to quantitate drug levels in the samples. Preparation of calibration standards, quality control samples and PK samples used a contemporary FDA bioanalysis document as a general guideline [17]. Drug concentration in the samples were derived by plotting reference standard sample concentrations versus 450 OD signal intensity in a 4-parameter fit using Softmax Pro software (Molecular Devices, CA, USA).

The ELISA method was used for all three sample types (serum, liquid whole blood and Mitra[®]). Concentration data obtained from liquid whole blood and Mitra[®] samples (C_{wb}) were divided by a factor based on male rat hematocrit of (42.30 ± 3.39)% [18,19] for serum equivalence comparison (C_{serum}) using the following equation with the assumption that there is no significant antibody drug uptake into blood cells (no published information about significant antibody uptake into blood cells):

$$C_{\text{serum}} = C_{\text{wb}} / (1 - 42.30\%)$$

Drug recovery from Mitra[®] micro sampler & liquid whole blood

Three concentrations of trastuzumab and daclizumab (0, 100 and 1000 ng/ml) were spiked into fresh rat whole blood to make whole blood samples.

Individual 20 µl aliquots of those samples (n = 3) were mixed into 180 µl of PBS immediately as the liquid whole blood group.

Individual Mitra[®] microsampler (n = 3) were dipped mid-way into each whole blood sample volume for 12 s, then transferred to the Mitra[®] clamshell containers where they were allowed to dry overnight at ambient temperature. The tip of each Mitra micro sampler was then removed into a 2-ml microtube by pushing the tip against the inside wall of the microtube and 200 µl of PBS were added. The microtubes were allowed to shake at 500 rpm at room temperature for 1 h and the samples were then spun down and transferred to fresh tubes for further bioanalysis. Each sample was evaluated in duplicate on the ELISA plate.

The equations below were used for drug recovery rate (RR) and relative standard deviation (RSD) calculations. C_b is back-calculated concentration; C_e is expected nominal concentration; SD is standard deviation; Average is the average concentration of duplicate samples.

$$RR = (C_b - C_e) / C_e \times 100\%.$$

$$RSD = SD / \text{Average} \times 100\%.$$

Rat pharmacokinetics study

Dosing & sampling

The study was reviewed and approved by the IACUC and conducted in full compliance with ethical and regulatory principles and local, national licensing regulations. The in-life study was conducted at Covance Laboratories Inc. (WI, USA).

Trastuzumab and daclizumab were administered to male Wistar rats ($n = 4/\text{group}$) via intravenous (iv.) and subcutaneous (sc.) administrations at 2 mg/kg. Approximately 0.15 ml of blood samples were sequentially drawn from a jugular vein at predose, 0.25, 2, 4, 8, 24, 48, 144, 168, 336 and 504 h post dose. Blood was then immediately transferred to either K₂EDTA-containing tubes (Mitra® and liquid whole blood samples) or serum separator tubes (serum samples). The blood in serum separator tubes was allowed to clot at ambient temperature within 1 h after collection prior to centrifugation to recover serum.

Sample processing

Three different sample processing methods were then applied:

- Mitra® dried blood samples:
Triplicate 20 µl aliquots were collected from each K₂EDTA-treated whole blood sample using three individual Mitra® microsamplers as follows: the blood sample tube was held at an angle and the collection tip of the microsampler was dipped midway into the sample for approximately 12 s. The process was then repeated for the remaining two aliquots. The Mitra® microsamplers were then placed into a clamshell package for drying and subsequent overnight shipment to the sponsor at ambient temperature. Further sample treatment was the same process as described in above section. The samples were then diluted according to ELISA quantitation range for bioanalysis.
- Liquid whole blood samples:
A single 20 µl aliquot of fresh whole blood was collected from the K₂EDTA-treated sample and transferred into tubes containing 180 µl PBS. The samples were mixed gently by inverting the tubes a few times. Liquid whole blood samples were then diluted according to ELISA quantitation range for bioanalysis.
- Serum samples:
Approximately 0.15 ml of whole blood was collected into serum separator tubes for serum. Serum samples were then diluted according to ELISA quantitation range for bioanalysis.

Results & discussion

Bioanalysis method matrix effect evaluation results

To ensure one generic ELISA method was appropriate for both whole blood and serum samples, experiment were conducted for evaluating whether there were matrix effects between whole blood and serum. Reference calibration standards of trastuzumab and daclizumab were made in final 10% rat whole blood, 10% rat serum and pure sample diluent. Each sample was analyzed in duplicate. Three standard curves of each drug overlaid very well (Supplementary Figures 1 & 2) and RSD was all below 5% (Supplementary Table 1). These results confirm that there was no significant matrix effect and that the same generic ELISA method could be applied for both whole blood and serum samples.

Drug recovery from Mitra® microsampler & liquid whole blood samples

Samples spiked with trastuzumab or daclizumab of 0, 100 and 1000 ng/ml were made in fresh rat whole blood. Triplicate Mitra® microsamplers were used to collect 20 µl of whole blood samples. For the liquid whole blood sample group, triplicate of 20 µl from the same whole blood samples were transferred to tubes containing 180 µl of PBS and then gently mixed by inverting the tubes a few times. Drug concentration was then quantitated for both sample types utilizing the ELISA method described previously. Most samples showed drug RR between 70 and 130% (Supplementary Tables 2 & 3). The drug recovery RSD between the Mitra® and liquid whole blood groups is below 20% for trastuzumab and below 14% for daclizumab (Supplementary tables 2 & 3).

Slightly lower trastuzumab RR was observed in liquid whole blood sample group comparing with Mitra® group, possibly due to the effect of whole blood hematocrit during drug spiking and dilution procedures. Mitra® microsampler therefore showed advantage over liquid whole blood sampling in that respect.

Table 1. Pharmacokinetics parameters (mean of four rats $\pm$ standard deviation) of terminal half-life (HL_{Lambda}), clearance (Cl_{obs}), mean residence time, volume of distribution at steady state and area under curve from 0 to 504 h (AUC_{0-504 h}) are listed to compare between the five different sample groups (serum, liquid whole blood and Mitra[®] -1, 2, 3) in trastuzumab 2 mpk iv. pharmacokinetics study.

Trastuzumab 2 mpk iv. (n = 4)	Serum	Whole blood	Mitra (average of 12)	RSD among three methods (%)	Mitra-1	Mitra-2	Mitra-3	RSD among Mitra triplicate (%)
HL _{Lambda} (h)	347 $\pm$ 64	300 $\pm$ 14	309 $\pm$ 80	7.9	336 $\pm$ 102	313 $\pm$ 97	278 $\pm$ 36	9.6
Cl _{obs} (ml/h/kg)	0.18 $\pm$ 0.02	0.17 $\pm$ 0.01	0.18 $\pm$ 0.02	2.9	0.17 $\pm$ 0.02	0.18 $\pm$ 0.02	0.19 $\pm$ 0.01	5.9
MRT (h)	447 $\pm$ 79	401 $\pm$ 17	404 $\pm$ 99	6.1	437 $\pm$ 130	413 $\pm$ 119	362 $\pm$ 35	9.5
V _{ss} (ml/kg)	81 $\pm$ 6	70 $\pm$ 4	70 $\pm$ 8	9.0	70 $\pm$ 9	71 $\pm$ 10	67 $\pm$ 6	2.8
AUC 0-504 h (h* μ g/ml)	7399 $\pm$ 359	8123 $\pm$ 484	8114 $\pm$ 408	5.3	8365 $\pm$ 204	8001 $\pm$ 296	7975 $\pm$ 595	2.7

AUC: Area under curve; MRT: Mean residence time; RSD: Relative standard deviation; V_{ss}: Volume of distribution at steady state.

Table 2. Pharmacokinetics parameters (mean of four rats $\pm$ standard deviation) of terminal half-life (HL_{Lambda}), maximum (C_{max}), mean residence time and area under curve from 0 to 504 h (AUC_{0-504 h}) are listed to compare between the five different sample groups (serum, liquid whole blood and Mitra[®] -1, 2, 3) in trastuzumab 2 mpk sc. pharmacokinetics study.

Trastuzumab 2 mpk sc. (n = 4)	Serum	Whole blood	Mitra (average of 12)	RSD among three methods (%)	Mitra-1	Mitra-2	Mitra-3	RSD among Mitra triplicate (%)
HL _{Lambda} (h)	340 $\pm$ 74	281 $\pm$ 56	276 $\pm$ 42	11.8	294 $\pm$ 42	291 $\pm$ 32	245 $\pm$ 42	9.9
C _{max} (μ g/ml)	19.7 $\pm$ 0.42	15.4 $\pm$ 0.76	14.7 $\pm$ 0.42	16.3	15.1 $\pm$ 0.84	14.3 $\pm$ 0.95	14.8 $\pm$ 1.44	2.9
MRT (h)	497 $\pm$ 96	441 $\pm$ 71	434 $\pm$ 55	7.6	451 $\pm$ 57	458 $\pm$ 42	392 $\pm$ 50	8.3
AUC 0-504 h (h* μ g/ml)	6055 $\pm$ 98	5440 $\pm$ 47	5178 $\pm$ 159	8.1	5304 $\pm$ 69	5105 $\pm$ 110	5125 $\pm$ 209	2.1

AUC: Area under curve; MRT: Mean residence time; RSD: Relative standard deviation; sc.: Subcutaneous.

Pharmacokinetics analysis of trastuzumab & daclizumab dosed in rats

Trastuzumab and daclizumab 2 mg/kg iv. and sc. rat PK studies were conducted at Covance Laboratories and samples were shipped back to sponsor for analysis. ELISA bioanalysis was performed to obtain drug concentration data from each time point from each animal. The drug concentrations from five different groups (serum, liquid whole blood and Mitra[®] triplicate) from four rats are consistent overall. Certara Phoenix 64 WinNonlin 7.0 software (NJ, USA) was applied to the concentration data for the estimation of PK parameters such as terminal half-life (HL_{Lambda}), clearance (Cl_{obs}), maximum concentration (C_{max}), mean residence time (MRT), volume of distribution at steady state (V_{ss}) and area under curve from 0 to 504 h (AUC_{0-504 h}). Similarly, PK parameters calculated from the concentration data showed good agreement as well, suggesting that the three different sample preparation methods are comparable. Mean PK parameters (average of four rats) are listed in [Tables 1–4](#) to compare among the three different sample groups (serum, liquid whole blood and Mitra[®]), and also among the Mitra[®] triplicate. Visual PK profile overlay of the five groups in the PK studies is shown in [Figures 1–4](#). The RSD of the PK parameters among the three sample groups are all below 20% ([Tables 1–4](#)).

Mitra[®] microsampler performance

The RSD of the drug PK parameters among the Mitra[®] triplicate are all below 10% ([Tables 1–4](#)). The PK profile overlay of Mitra[®] triplicate performance in four animals are shown in [Supplementary Figures 3–6](#). The four animals in the daclizumab sc. PK study showed significant drug level differences in the last timepoint ([Supplementary Figure 6](#)), suggesting possible anti-drug antibodies (ADA) in some rats. The low RSD among the Mitra[®] triplicate groups demonstrated good performance of this approach.

Conclusion

Mitra[®] data obtained from this work demonstrated the potential and advantage of this method over whole blood and serum sampling in improving the three Rs in large molecular drug development in small animals. The

Table 3. Pharmacokinetics parameters (mean of four rats $\pm$ standard deviation) of terminal half-life (HL.Lambda), clearance (Cl.obs), mean residence time, volume of distribution at steady state, and area under curve from 0 to 504 h (AUC_{0-504 h}) are listed to compare between the five different sample groups (serum, liquid whole blood and Mitra[®] -1, 2, 3) in daclizumab 2 mpk iv. pharmacokinetics study.

Daclizumab 2 mpk iv. (n = 4)	Serum	Whole blood	Mitra (average of 12)	RSD among three methods (%)	Mitra-1	Mitra-2	Mitra-3	RSD among Mitra triplicate (%)
HL.Lambda (h)	211 $\pm$ 66	196 $\pm$ 69	193 $\pm$ 51	4.7	199 $\pm$ 64	203 $\pm$ 56	178 $\pm$ 44	6.9
Cl.obs (ml/h/kg)	0.24 $\pm$ 0.02	0.27 $\pm$ 0.05	0.27 $\pm$ 0.04	6.9	0.26 $\pm$ 0.05	0.27 $\pm$ 0.04	0.27 $\pm$ 0.04	2.4
MRT (h)	292 $\pm$ 79	270 $\pm$ 86	266 $\pm$ 59	5.1	269 $\pm$ 69	277 $\pm$ 69	251 $\pm$ 51	5.0
Vss (ml/kg)	69 $\pm$ 13	71 $\pm$ 10	69 $\pm$ 8	1.4	68 $\pm$ 10	72 $\pm$ 7	68 $\pm$ 8	3.1
AUC 0-504 h (h* μ g/ml)	6846 $\pm$ 366	6210 $\pm$ 557	6380 $\pm$ 558	5.1	6512 $\pm$ 711	6306 $\pm$ 542	6321 $\pm$ 551	1.8

AUC: Area under curve; MRT: Mean residence time; RSD: Relative standard deviation; Vss: Volume of distribution at steady state.

Table 4. Pharmacokinetics parameters (mean of four rats $\pm$ standard deviation) of terminal half-life (HL.Lambda), maximum (Cmax), mean residence time and area under curve from 0 to 504 h (AUC_{0-504 h}) are listed to compare between the five different sample groups (serum, liquid whole blood and Mitra[®] -1, 2, 3) in daclizumab 2 mpk sc. pharmacokinetics study.

Daclizumab 2 mpk sc. (n = 4)	Serum	Whole blood	Mitra (average of 12)	RSD among three methods (%)	Mitra-1	Mitra-2	Mitra-3	RSD among Mitra triplicate (%)
HL.Lambda (h)	187 $\pm$ 68	197 $\pm$ 51	169 $\pm$ 49	7.6	167 $\pm$ 56	165 $\pm$ 56	175 $\pm$ 60	3.2
Cmax (μ g/ml)	17.7 $\pm$ 1.84	14.9 $\pm$ 0.76	17.0 $\pm$ 0.23	9.0	16.7 $\pm$ 1.95	17.2 $\pm$ 2.54	17.0 $\pm$ 2.80	1.4
MRT (h)	272 $\pm$ 111	265 $\pm$ 97	251 $\pm$ 82	4.1	247 $\pm$ 75	248 $\pm$ 89	259 $\pm$ 104	2.8
AUC 0-504 h (h* μ g/ml)	5326 $\pm$ 87	4813 $\pm$ 163	4913 $\pm$ 188	5.4	4986 $\pm$ 161	4929 $\pm$ 186	4823 $\pm$ 227	1.7

AUC: Area under curve; MRT: Mean residence time; RSD: Relative standard deviation.

Figure 1. Drug concentration–time plots of three sample groups (serum, liquid whole blood and mean of Mitra[®] triplicate) for trastuzumab in rat iv. pharmacokinetics study.

Figure 2. Drug concentration–time plots of three sample groups (serum, liquid whole blood and mean of Mitra[®] triplicate) for trastuzumab in rat sc. pharmacokinetics study.

Figure 3. Drug concentration–time plots of three sample groups (serum, liquid whole blood and mean of Mitra[®] triplicate) for daclizumab in rat iv. pharmacokinetics study.

Figure 4. Drug concentration–time plots of three sample groups (serum, liquid whole blood and mean of Mitra[®] triplicate) for daclizumab in rat sc. pharmacokinetics study.

consistency and low RSD among the five groups of data demonstrates that Mitra[®] microsampling results are equivalent to those derived from both serum and liquid whole blood sampling with the advantages the other two methods do not possess. For example, only 10 or 20 μ l whole blood is taken by Mitra[®] approach while double the amount of whole blood is needed for serum sampling, and no clotting, centrifugation, freezing/refrigeration and dry/blue ice shipping/storage are needed for the Mitra[®] approach offering potentially significant advantages for long distance collaborations.

Additionally, by utilizing smaller sample volume for PK analysis, the Mitra[®] approach permits additional volume for other analyses such as biomarker profiling, thereby avoiding inter-animal variability associated with a composite sampling approach in small animals.

Future perspective

For the two mAbs evaluated in this work, drug stability after overnight shipping and drying at ambient temperature on Mitra[®] samplers was tested by drug concentration recovery by ELISA analysis. Drug stability for more than 24 h was not evaluated in this work. In a recent publication, a brief stability evaluation of a peptide was carried out for 48 h at room temperature showing 0.1% decrease after Mitra[®] microsampling and 11.6% decrease after DBS [20], indicating at least the short-term stability associated with Mitra[®] sampling for protein therapeutics. In the future, we would like to conduct further experiment to evaluate drug stability over longer period of time on Mitra[®] samplers. It is important that industry begin to gather and compare real-world Mitra[®] data to better understand both the advantages and limitations of this technology.

At the time when the rat PK was conducted, whether Mitra[®] microsampling could be achieved from tail vein directly by CRO staff was not confirmed. Therefore, in the rat PK study reported in the present work, Mitra[®] samples were taken from a tube containing freshly collected whole blood rather than directly from the rat tail. Since three different sampling methods were selected to be compared, rat was chosen for sampling volume requirement. In the future, as more research staff is trained with Mitra[®] microsampling, we would like to conduct a mouse PK study with direct tail Mitra[®] sampling to evaluate if similar data would be observed.

Summary points

- Three different sampling methods were compared in this paper: serum, liquid whole blood and Mitra[®] microsampling.
- Mitra[®] microsampling is convenient, requires no refrigeration or special care during storage or shipping, and adopts relatively simple drug extraction procedure.
- A rat pharmacokinetics (PK) study of trastuzumab and daclizumab was conducted with the three sampling methods.
- Drug levels retrieved from three different sampling methods were obtained and compared. Three data sets are in good agreement.
- The PK analysis from three sets of drug level data was conducted and compared. Three PK datasets are in good agreement.
- Among Mitra[®] triplicate groups, the data showed little variability. Mitra[®] has potential to be a convenient and reliable tool for large molecule analysis in early research animal studies while reducing and refining animal usage.

Supplementary data

See online at: <https://www.future-science.com/doi/10.4155/bio-2018-0228>

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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