Dried Blood Spot Analysis and its Application to Reproduction Toxicity Testing of Pharmaceuticals

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Abstract

The regulatory need to characterise exposure to the test item is well established and inclusion of kinetic endpoints on reproduction toxicity studies has intensified over the last decade. In parallel, the application of dried blood spot (DBS) technology for exposure assessment of pharmaceuticals has also gained growing industry interest due to its considerable advantages over traditional methods of bioanalysis. An industry shift is beginning and with successful regulatory submissions now being supported by DBS exposure data, more drug developers are starting to adopt the use of DBS analysis as their preferred bioanalytical method for their development programmes. The possible applications of DBS analysis on reproduction toxicity studies are discussed in this poster, highlighting the potential to revolutionise the evaluation of both maternal and offspring exposure data, by the inclusion of more comprehensive kinetic assessments whilst reducing cost and overall animal burden. Due to the minimal blood volume required for DBS analysis, additional time points and occasions for exposure assessment can now be realistically included into developmental toxicity studies, allowing serial profile evaluations without the inclusion of satellite groups. DBS technology also enables the collection of individual foetal exposure data, negating the need for total litter blood pooling. In addition, non-terminal serial profile data can now be obtained from the pre-weaning juvenile rat pup. Aside from the clear financial advantages achieved from the simplicity of DBS sample collection and room temperature sample storage and shipment, the refinement in the data quality that DBS analysis presents would in itself be a significant improvement. When one considers, however, the considerable reduction in the numbers of animals required, it is easy to see how the use of DBS analysis is attractive. Given the preference to maintain the use of one biological matrix for exposure assessment throughout the clinical and non-clinical drug development programme, it should be expected that the future use of DBS analysis will be required on reproduction toxicity development programmes, thus providing the opportunity to use this technique in these studies and benefit from the unequivocal advantages it can offer.

Introduction

The need to characterise exposure to the test item during reproduction toxicity studies is well established given the known potential for altered ADME properties (1) and determinations of both maternal and offspring exposure have intensified over recent years. Plasma has been the traditional matrix of choice however, this entails further sample processing after collection and larger initial blood volumes. Interest in the development of DBS technology as an alternative matrix has arisen due to the considerable advantages this technology can offer (2, 3). Namely, simplified sampling and storage procedures in both the clinical and non-clinical setting, increased storage stability in some circumstances (4), the use of minimal volumes of whole blood (10 µL to 40 µL) and the resultant substantial reduction in cost, animal burden and test item usage. Overall these advantages offer huge contributions to the application of the three R's (Reduction, Refinement and Replacement) and over the last few years Sequani has embarked on a number of validation studies to investigate practical techniques and the suitability of DBS analysis results (5, 6, 7).

A number of drug developers are now beginning to adopt DBS analysis as their preferred biological matrix in early development. Given the understandable preference to maintain one matrix throughout the development programme, it is inevitable that reproduction toxicity studies will soon also be adopting DBS analysis. This poster will therefore explore how to best maximise the advantages this new technology can offer for toxicokinetic evaluations on reproduction toxicity studies.

DBS analysis in the pregnant or lactating rodent

In comparison to traditional plasma bioanalysis, from a scientific and ethical perspective, the largest contributions that DBS analysis can offer are the refinements in the quality of data obtained and the reduction in the numbers of animals or litters required to achieve the data, therefore providing opportunities for more robust assessments and meaningful interpretation.

The maximum volume of blood which can be obtained from one animal before it must be euthanised is limited by established industry guidance (8). Blood volumes required for plasma analysis are variable depending on the sensitivity requirements of the method and the number of analytes to be examined, however, in general, blood volumes are rarely less than 0.3 mL whole blood and, on occasion, significantly larger volumes are required, particularly where the test item is a pro-drug. For DBS analysis, the minimal blood volume required for one analytical run is just 10 µL, with 40 µL providing up to 4 repeat analyses. Whilst blood volumes are rarely limiting in larger animal species, toxicokinetic evaluations in rodents frequently require the inclusion of satellite animals due to the potential physiological disruption caused by hypovolemia and the understandable hesitance to perturb the main study animals. Furthermore, in many instances where repeat sampling occasions are required, terminal sampling may be needed and almost exclusively, due to blood volume limitations, toxicokinetic data are derived from composite profiles. This is an accepted compromise but it is not ideal and overall a greater number of animals are required to achieve less optimal data.

When compared with plasma analysis, DBS analysis presents a clear advantage for all rodent studies; however, the need to minimise the disruption to the animal is all the more important when the animal is pregnant or nursing offspring. By the use of DBS analysis, the blood sampling procedure, which typically uses the lateral tail vein, can be less invasive. Moreover, whilst anaesthesia of pregnant animals is usually avoided, it is not always possible when large blood volumes are required; DBS analysis would eliminate this scenario. Furthermore, due to the minimal volumes required, the pregnant or lactating animal could realistically have serial blood samples collected on multiple occasions without the need for terminal sampling or the risk of hypovolemia.

Table 1 Impact of plasma or DBS analysis on adult animal usage and blood volume

	Number of time points per occasion*	Total blood volume sampled from each animal (Percentage of circulating blood volume)	Total number of animals required number per group per sex)
Plasma analysis	6	2 mL (15 %)	48 (6)
DBS analysis	6	0.48 mL (3.7%)	24 (3)

*Assumes two occasions for TK evaluation and rats of at least 200 g body weight

The use of DBS analysis also presents exciting opportunities for blood sampling in lactating females. Given that the largest differences in maternal and offspring exposure can be during the early post-natal period (1), it is not surprising that a growing number of development programmes include both dam and pup exposure assessments of this nature. Whilst full toxicokinetic profiles may not always be required from the dam, this is now a more realistic option where DBS analysis is employed, thus providing greater flexibility. Multiple occasions throughout the lactation period could now be investigated without causing a significant disturbance to the physiological condition of the dam and given that on some occasions, significant test item concentration differences in the blood compared to the milk can occur throughout lactation, these data could facilitate more accurate estimates of exposure risk (9). Whilst only a small blood volume is collected from the dam, it should, however, be considered that serial sampling would remove the nursing dam from the litter on a greater number of occasions. Clearly the benefit of this additional exposure information should outweigh the risk to the nursing of the offspring, and on occasions where relatively intensive sampling from the lactating female is included, the inclusion of satellite groups may still be advisable.

Due to the rarity of the use of the mouse as the rodent of choice for reproduction toxicity testing, this poster has focused on rat sampling. It should be noted however that due to the large animal burden posed by mouse toxicokinetic evaluations, Sequani has conducted numerous validations of mouse DBS analysis (5) establishing techniques to serial sample mice on up to 6 occasions. The use of DBS analysis in mouse reproduction toxicity testing is, therefore, feasible and one could argue that in this instance, there is an even greater incentive.

DBS analysis in the offspring

The advantages to the use of DBS analysis in the pregnant or lactating rat have been discussed but it could be argued that the largest benefit this technique has to offer is in offspring exposure assessments.

Blood sampling from juvenile pups is clearly problematic with traditional blood sampling techniques largely impractical for the pre-weaning rat. This is further complicated by limitations in the total circulating blood volume. Despite this, where there is known risk for milk or placental transfer, characterisation of the exposure is useful, particularly during the vulnerable period shortly after birth when clearance systems are especially poor. This has resulted in the typical use of terminal blood sampling techniques, such as decapitation, increasing the number of animals required to generate data which can often be limited to a pooled blood sample. Using plasma bioanalysis this can amount to just proof of absorption, with no toxicokinetic profiling possible and, where additional time points are required for evaluation, this often entails the sampling of an entire litter for each occasion.

In the absence of existing methodology, Sequani has validated a non-terminal juvenile rat blood sampling technique suitable for use with DBS analysis. Due to the minimal blood volume required, blood samples, suitable for at least one analysis, and on multiple occasions for repeat analysis, were successfully collected from the ventral tail artery of Sprague Dawley rat pups. Sampling was non-invasive and was not associated with decline in growth or clinical condition (See Figures 1 to 3). Furthermore, serial sampling feasibility was established and from the same animals, samples were collected on up to 4 occasions on Day 7 of age and on up to 6 occasions on Day 14 of age (7).

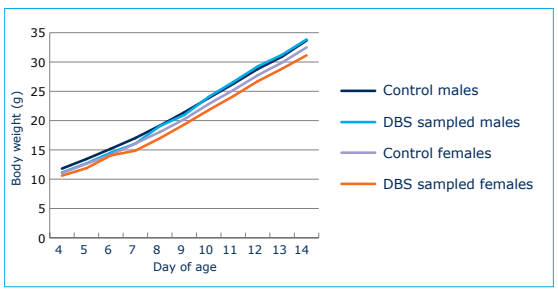

Figure 1 Body weight comparisons between DBS sampled juvenile animals and Controls

Figure 2 Ventral tail artery sampling on Day 7 of age

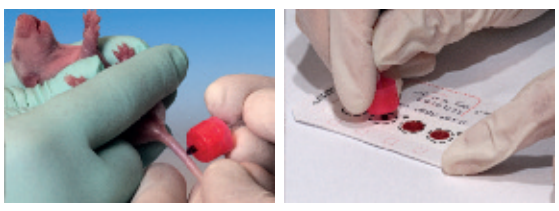

Figure 3 Blood collection and spotting on Day 7 of age

By adopting DBS analysis, it now becomes a credible option to include multiple toxicokinetic evaluations in pups from pre- and post-natal studies. Not only would the data be more robust by the use of serial profiles, but the potential exposure differences throughout the pre-weaning period could be fully characterised from multiple animals at each time point, allowing more comprehensive safety evaluation. It is especially compelling that this could be obtained from non-terminal methods resulting in a huge reduction in the numbers of pups used and by retaining very few additional pups within the litter after culling, this could eliminate the need for any additional litters.

Table 2 Impact of plasma or DBS analysis on pup usage and blood volume

	Number of time points per occasion*	Total blood volume sampled from each animal (Percentage of circulating blood volume)	Total number of animals required number per group per sex)
Plasma analysis	6	0.3 mL – Maximum obtainable blood volume sampled terminally	144 (18)
DBS analysis	6	120 µL (12.5%)	24 (3)

*Assumes one occasion for TK evaluation and rats of at least 15 g body weight

The full haematological impact of serial sampling from such a young age during development is, at this stage, undetermined. Consequently, Sequani are also embarking on additional validation work to fully investigate this; however, given the minimal volume of blood sampled, and the absence of any adverse effects of sampling during the preliminary validation work, it seems unlikely that there are any significant negative outcomes to sampling in this way. In this situation it could also be argued that toxicokinetic sampling could be conducted on main study animals, further decreasing animal usage.

The use of DBS could also be of benefit for foetal blood sampling. Typically, due to the small blood volume achievable from the foetus, even by decapitation, pooling of blood samples is routine. By using DBS analysis, it is a viable option that one foetus may provide one sample for analysis. Clearly terminal sampling is unavoidable; however, by avoiding the pooling of blood, information on exposure differences along the uterine horn may be obtained and could in some circumstances, aid in the interpretation of the foetal abnormality data. Furthermore, it is probable that from just one foetus, sufficient blood would be collected to allow repeat analyses.

One perceived disadvantage relating to DBS analysis is related to its potential inability to detect very low circulating blood concentrations. Evidently there is a greater potential risk in foetuses and juvenile pups that are not dosed directly, but it should be considered that solvent extraction can be conducted on multiple blood card discs in order to increase signal strength, although clearly this could affect availability for repeat analyses. This is really an issue of analytical sensitivity and with modern instrumentation there is considerable scope for improvement in limits of quantitation.

Conclusion

Toxicokinetic data derived from whole blood have long been acceptable to the regulatory authorities and in some circumstances, by removing assumptions about drug binding to the cellular fraction, whole blood derived data can be more robust. The use of DBS analysis, utilising whole blood as an alternative biological matrix, is now becoming a realistic option. Given the preference to maintain one biological matrix throughout the development programme, and the growing use of DBS analysis in the earlier non-clinical studies, it should be expected that DBS analysis will soon also be required for the reproduction toxicity programmes, enabling the exploitation of the clear advantages this technology can offer. It now becomes feasible to serially sample the same animal to provide full toxicokinetic profiles without compromising even the pregnant or lactating animal and negating the need for additional satellite groups. Not only is this possible for multiple occasions in the dam, but also for the offspring. This technology has revolutionising potential, presenting a superior approach in terms of ethics, cost and quality of data.

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Methods for DBS analysis

- A small whole blood volume (between 10 µL to 40 µL), is collected into an EDTA coated capillary tube (See Figure 4)
- Using a specifically manufactured DBS card (for example, Whatman DMPK – A cards), the capillary tube is placed above the card to allow blood to flow from the tube onto the card. (See Figure 3)
- The DBS cards are made from specially manufactured filter paper designed to allow the blood to saturate the paper creating a "blood spot", whilst giving an even and reproducible spread.
- Blood spots are allowed to dry at room temperature for approximately two hours.
- After drying, cards can be stored with desiccant and shipped at room temperature or analysed.
- For analysis, a small disc (3 mm diameter) of blood saturated card is punched out and analytes are typically recovered by solvent extraction. Multiple discs can be combined to increase signal strength if required (a blood volume of 10 µL is sufficient to give one blood spot, See Figure 5)
- Samples can then be analysed by LC-MS/MS, using conventional methods

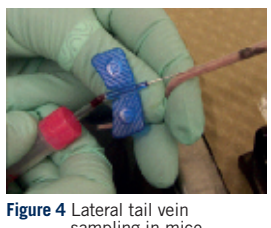

Figure 4 Lateral tail vein sampling in mice

Figure 5 Blood saturated disc collection

Example results

Validation study results (5, 6), assessing any differences between DBS or whole blood toxicokinetic data, are presented below:

Table 3 Mean Toxicokinetic parameters of parent and metabolite

Matrix	Whole Blood		Blood Spot	
Analyte	Parent	Metabolite	Parent	Metabolite
Cmax *(ng/mL)*	4330	42.0	4490	39.9
Tmax *(hr)*	1	2	1	2
AUC0-tlast *(ng.hr/mL)*	9140	211	9790	217
Whole Blood vs. Blood Spot *(Cmax)*	1.0	1.1		
Whole Blood vs. Blood Spot *(AUC0-tlast)*	0.9	1.0		

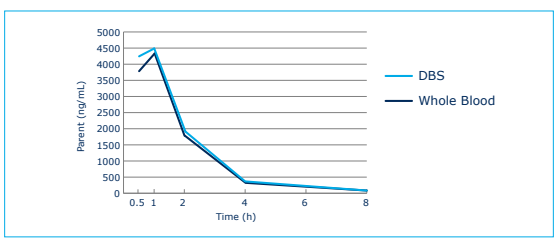

Figure 6 Mean Analyte Profile of Parent (DBS and Whole Blood Analysis)

Good concordance was demonstrated between both maximal and systemic exposure data, indicating reliable results can be obtained from DBS analysis.