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Overcoming the barriers to the uptake of nonclinical microsampling in regulatory safety studies☆

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Toxicokinetic analysis is an essential part of nonclinical drug development. Advances in bioanalytical techniques have opened up the potential to use smaller sample volumes (microsamples) to assess drug exposure in blood, plasma and/or serum. Microsampling can increase the amount of nonclinical safety information available, improve its validity by linking toxic effects to drug exposure in individual animals and represents the most significant opportunity to reduce animal use in toxicology studies in the short term. In May 2013, a workshop was held with 80 delegates from 33 companies with the aim of sharing information and knowledge on microsampling technologies. This article covers the discussions at the workshop, current practice in the industry, regulatory experiences and the future direction of microsampling across drug development.

Introduction

During the development of new drugs, nonclinical safety studies are carried out in rodent and non-rodent species to identify and characterise adverse effects and facilitate risk assessment for clinical studies. Toxicokinetic (TK) data are an essential component of these studies and are used to correlate circulating drug concentrations (exposure) with pathology or functional effects, the primary endpoints in safety assessment studies. The matrix for determining drug concentration could be blood, plasma or serum.

Exposure–response correlations in animals are subsequently used to define a safe starting dose level in the clinic to set stopping rules and avoid harmful drug accumulation or interactions. Requirements for safety assessment and TK are described in international regulatory guidelines issued by the International Conference on Harmonisation (ICH) [1]. The guidelines indicate that TK information should be obtained to provide proof of drug exposure during the period of dosing but do not dictate how exposure is measured, thereby enabling technological innovations in the bioanalysis of TK samples. TK data are used in a number of ways. For example, it is important to know if there is accumulation over time or if the active drug is clearing differently after repeated drug doses, as might happen with metabolic induction, immunogenic clearance or intoxication of target organs involved in clearance. Exposures are also examined across dose levels to determine whether drug concentrations increase proportionally with the delivered drug dose or if there are irregularities as a result of altered drug absorption or compartmental saturation. Differences in exposure between males and females can also be investigated. All of this information is important for decision making on dosing routes and frequencies in a given patient population.

Individual TK blood samples are typically required at four to six timepoints (within a 24 hour period for small-molecule drugs and over several days for biopharmaceutical drugs) on at least two occasions in each nonclinical safety study (Table 1). Conventionally, a blood volume

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TABLE 1

An example study design for toxicokinetic (TK) analysis on a one month good laboratory practice (GLP) rat study

Animal number	Sampling timepoint					
	#1	#2	#3	#4	#5	#6
1	x			x	x	
2	x			x		x
3		x		x	x	
4		x	x			x
5	x		x	x		
6	x		x		x	
7	x		x			x
8		x			x	x
9		x	x		x	
10		x		x		x
	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$

All the main study animals are sampled. There are a total of ten animals per sex per group (80 rats). TK profiles are made up from composite samples as follows: six timepoints (#1 to #6), three samples per animal (see x in rows), five samples per timepoint (see x in columns). This gives a total of 30 TK samples per sex per group. Previously 18 samples per sex per group were taken sampling satellite animals (an additional 18 rats).

of $\geq$200 μl has been required to determine circulating drug concentrations. In rodent studies, this relatively large volume of blood could cause anaemia or other secondary effects such as bone marrow and haematological changes, which would confound interpretation of primary drug effects. Therefore, these blood samples are often taken from satellite animals, which are added to the study solely for TK purposes. This can lead to a large increase in the number of rodents required for a typical study. For example, for a typical 4 week repeat oral dose rat study an additional three to nine satellite animals per dose group per sex might be required (depending upon the sample volume and number of timepoints required) in addition to ten main study animals. Further, the use of TK satellite animals means that there is no way to correlate drug levels directly with drug action, because pathology or functional effects are measured in the main study animals and drug exposure is measured in similarly dosed TK satellite animals. The blood, plasma or serum is typically analysed by either LC–MS/MS, or immunoassay to determine levels of drug and, in some cases, associated metabolites, anti-drug antibodies (ADAs) or pharmacodynamic (PD) endpoints. The focus of this paper is on the evaluation of small molecules in rodents because this is where current microsampling efforts are directed and are likely to have the biggest impact. However, the benefits and use of microsampling are not limited to this area. It is often assumed that the rodent is not a relevant model for 'biologic' drugs such as monoclonal antibodies (mAbs), but the screening for potency and use of the rodent for mAbs is on the rise and expected to increase further. Additionally, blood sample volume in rodents and larger species, such as non-human primates, is particularly challenging for biologics because these often require sampling for determination of TK and ADAs as well as monitoring of PD endpoints [ICHS6 (R1)].

The collection of samples for TK analysis using conventional rodent study designs (i.e. with satellite animals) has been identified as the largest influence on rodent numbers used in regulatory toxicology studies [2]. Therefore, microsampling represents the most significant opportunity to reduce rodent use in toxicology studies in the near term. Advances in the sensitivity of bioanalytical techniques, particularly LC-MS/MS, now provide the capability to conduct analysis with much smaller volume samples, around 25–30 μl; these are termed 'microsamples'. A number of the approaches currently in use are illustrated in Fig. 1 [3–6]. The potential benefits of microsampling are just beginning to be realised in drug development and could have profound effects on regulatory safety assessment studies. This article discusses the barriers to the more widespread adoption of microsampling and outlines the ways forward based on the output from a recent meeting organised by the NC3Rs, an independent scientific organisation that drives innovative technologies to replace, reduce and refine the use of animals in research and safety testing.

Benefits

There are scientific, business and animal welfare benefits to employing microsampling in association with sensitive assay technology. From a scientific perspective, TK sampling from main study animals allows direct correlation of functional and/or pathological changes with concentration of test article in the individual animal's blood. This allows a clear connection between drug exposure and drug action, as is currently the norm in larger preclinical species and human patients but rare in standard rodent studies. Microsampling in all species can also provide scope for the use and characterisation of a broader array of biomarkers, enabling better insight into pharmodynamic effects. The aim is that these endpoints will facilitate better research translation and help to mitigate risks in clinical trials through improved clinical monitoring. From a business perspective, when reduced numbers of satellite animals or none at all are used, less compound and resource (dosing, handling and care) is needed and this can potentially lead to notable cost savings for these studies. There is also significant potential to refine blood sampling procedures across all species to make collection of samples quicker and less stressful for the animal than conventional sampling. In addition to improved welfare, this will deliver better science in that there would be less disturbance to critical physiological parameters (e.g. heart rate and respiratory rate). These wide-ranging incentives have contributed to making microsampling a hot topic of debate within the industry.

The challenge

In May 2013 in central London, the NC3Rs hosted a workshop for 80 delegates from 33 companies and representatives from regulatory bodies to share information and knowledge on the novel microsampling technologies being used and what the barriers were to further implementation. All companies provided information on their current use of, and future plans for, microsampling within drug development through a pre-meeting questionnaire. In identifying barriers to the uptake of microsampling techniques, it was established that there are two primary aspects: (i) functional and clinical pathology evaluation and (ii) approaches to bioanalysis and TK. To date, much of the debate has centred on bioanalytical methods and whether the assay will deliver enough sensitivity with the small samples available and it has been established that many of the bioanalytical issues are surmountable. Much less attention has been given to the real or perceived issues regarding

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