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Assessment of toxicological effects of blood microsampling in the vehicle dosed adult rat

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ABSTRACT

Historically, satellite groups are often used for rodent toxicokinetic profiling because of the haematological consequences of blood sampling. If microsampling is shown to be toxicologically benign, its adoption in rat studies would enable comparison of exposure and toxicity in individual animals (as happens in non-rodent studies) as well as obviating need for satellite groups.

Methods: Groups of 10 male (200–300 g) and female (150–250 g) rats aged 10 weeks were vehicle dosed and either left unsampled, conventional blood volume sampled (6 × 200 μL) or microsampled (6 × 32 μL) on Days 1 and 14. At termination on Day 15, clinical pathology plus liver and spleen weights and histopathology were obtained.

Results: All clinical pathology parameters were within background range. However, compared to unsampled controls, conventional volume sampled rats showed a statistically significant ($p < 0.001$) decrease in haemaglobin, haematocrit and red blood cell count, an increase in reticulocytes (at least $p < 0.01$), increased AST and GLDH and, in males only, an increase in monocytes and neutrophils. In contrast, microsampled animals showed no changes except for a slight, toxicologically insignificant decrease in haemoglobin concentration (15.0 g/dL compared to the unsampled group mean of 14.4 g/dL) in females ($p < 0.05$) and a small increase in monocytes ($p < 0.05$) in males.

Conclusion: Microsampling of adult rats is possible without adverse toxicological consequences.

1. Introduction

During the development of new medicines, non-clinical safety studies are carried out in rodents and non-rodents to identify and characterise adverse effects and facilitate risk assessment for clinical studies in humans. Toxicokinetic data are an essential component of non-clinical studies and are used to correlate circulating drug concentrations (exposure) with functional or pathological changes in the animals. Requirements for safety assessment and toxicokinetics are described in international regulatory guidelines and indicate that toxicokinetic information should provide proof of drug exposure during the dosing period, but do not dictate how exposure is measured, thereby allowing for technological innovations in bioanalysis (ICH, 1994).

The collection of samples for toxicokinetic assessment using rodent study designs with satellite groups has been identified as the largest influence on rodent numbers used in non-clinical safety studies (Sparrow et al., 2011).

In general, satellite animals are used for in-life blood sample collection for toxicokinetic profiling in rodent general toxicology studies. The most common species of rodent used in general toxicology studies is the rat. Provided it is shown to have minimal toxicological consequences, microsampling of main test animals in rodent toxicity studies, for toxicokinetic profiling, could remove the need for satellite animals and allow the link between exposure and toxicity to be compared within individual animals, as is the general situation for non-rodent studies.

Historically, there has been a reluctance to use main test rats for toxicokinetic exposure monitoring. From a toxicological perspective, this reluctance was based on the concern that the volume of blood taken and/or the blood sampling procedure itself could physiologically compromise the rat such that the toxicological profile of the test compound could be rendered more difficult to interpret. The lack of specific data demonstrating the magnitude of effect of different blood sampling regimes on critical toxicological endpoints has hampered the adoption of toxicokinetic monitoring of main test rats on toxicology studies. In previous publications there is evidence eluding to the lack of effect on clinical pathology parameters after blood sampling (Jonsson et al., 2012, 2013) but a definitive assessment of these parameters in vehicle dosed rats

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under controlled conditions has not been previously reported. In order to address this data gap, an experiment was designed in which toxicological endpoints that were deemed likely to be sensitive to blood sampling (e.g. haematology, clinical chemistry plus liver and spleen weights and histopathology of liver and spleen) were compared across groups of rats that were either unsampled or had 6 time point sampling regimes using either conventional (200 uL) volume or microsample (32 μL) volumes. The sampling regime was designed to represent a typical 6 timepoint toxicokinetic profile. The animals were vehicle dosed daily for 14 days with blood sampling on Day 1 and 14 prior to termination on Day 15. Corticosterone was added into the clinical chemistry panel as an indicator of stress.

In addition to the primary purpose of exploring the impact of blood sampling on sensitive toxicological endpoints, the following technical elements were also explored:

To assess the duration of warming of animals in a 38 °C hot box, required to enable conventional volume sampling or microsampling.
To assess type of restraint required for microsampling.
To isolate plasma from all microsamples in order to assess the logistical feasibility of taking and processing a relatively large number of capillary tube microsamples in quick succession.

2. Material and methods

2.1. Animals

Sixty adult Crl:WI(Han) rats (30 Males and 30 Females) approximately 10 weeks of age (Males: 200–300 g; Females: 150–250 g) were used on the study split into Groups 1, 2 and 3. Group 1 was assigned to be an unsampled 'control' group, Group 2 a conventional volume sampled group and Group 3 a microsampled group. Each group contained 10 animals per sex, with animals randomised to cages based on initial bodyweight. A group size of ten was chosen to match the group size used on one month pivotal toxicity studies. Animals were housed five per sex per cage with groups assigned to cages based on a latin square design. Animals were kept under standard conditions e.g. in solid bottom tinted plastic cages (Techniplast 2000P), with woodchip bedding, room maintained at temperature 21 °C ± 2 and humidity 55% ± 15 on a 6 am–6 pm light cycle. Water from the site drinking water supply and RM1 (E) SQC pelleted diet supplied by Special Diets Services Ltd., England, was freely available. Chew sticks, nesting material and tunnels was provided for environmental enrichment.

2.2. Clinical observations

All animals were thoroughly examined for clinical signs after arrival and had a subsequent acclimatisation period of one week. All animals were also inspected for clinical signs at least twice daily, observations were recorded, and a physical examination was done at least once weekly. Body weights of all adult animals were recorded at randomisation and then twice weekly from Week-1 (including Day 1) to termination. Food consumption was recorded twice weekly during the vehicle dosing period (including Day 1) on the same days as bodyweight was recorded.

2.3. Vehicle dosing

All animals were vehicle-dosed with 5 mL/kg water containing 0.5% w/v hydroxypropyl methylcellulose and 0.1% w/v polysorbate 80 by oral gavage in the morning between 8 am and 12 pm for 14 Days prior to termination on Day 15 after the 24 h toxicokinetic sample was taken.

2.4. Blood sampling for sham toxicokinetic profiling

A six hour time point blood sampling regime on Day 1 and Day 14 of vehicle dosing was followed (0.5, 1, 2, 4, 8 and 24 h), designed to be typical for a regulatory study (see Table 1). Tail vein blood sampling was either by a conventional (200 μL Group 2) volume sampling procedure or by a microsampling (32 μL Group 3) procedure. A concurrent un-sampled vehicle control group (Group 1) was included in the study design.

Prior to in-life sampling all animals to be sampled were warmed in a 38 °C hot box to encourage vasodilatation to aid removal of venous blood. Time in the hot box differed depending on sample size to be removed. In brief, animals that were to be microsampled (32 μL) were warmed for 1–2 min prior to sampling. All other animals that were to be conventional volume sampled (200 μL) were warmed for between 5 and 13 min (most commonly approximately 10 min) prior to sampling. Concurrent vehicle control animals were not placed in a hot box or sham handled.

2.4.1. Restraint for sampling

This study attempted to use the least restraint for taking the sham toxicokinetic blood samples. For conventional and micro - sampling of blood, animals were firstly not restrained and were placed on the arm of the technician for sampling. If that was unsuccessful, the animals were lightly restrained (i.e. held in the hand). Standard tube restraint was used as a last resort if the other two methods were unsuccessful. Two technicians were required for blood sampling if animals were hand held, one or two technicians for unrestrained animals and just one technician if the standard tube restraint method was used.

2.4.2. Conventional volume sample collection

Approximately 200 μL of blood from Group 2 animals was collected from the tail vein into K_2EDTA coated tubes using a 25G needle, dripping the blood into the tube. 200 μL is a volume typically used for toxicokinetic blood sampling in this facility on regulatory studies. All blood samples were gently mixed. Plasma was prepared within 30 min of blood sampling by centrifugation at 1500*g* for 10 min at approximately +4 °C. After centrifugation, the plasma was transferred into plain polypropylene tubes. These sham toxicokinetic samples were not analysed.

2.4.3. Microsample collection

Approximately 32 μL of blood from animals in Group 3 were collected using a toxicokinetic microsampling procedure (Jonsson et al., 2012). Briefly, this methodology required two technicians, one animal handler and one to take the blood sample. Using a 21G needle a puncture was made in the tail vein, a volume of 32 μL whole blood was collected at each sampling time point using haematocrit tubes (K_2EDTA treated). The haematocrit tube was plugged in one end with wax and stored on ice until separated by centrifugation for plasma isolation.

Plasma was prepared within 30 min of blood collection by centrifugation (1500*g*, 10 min, +4 °C). After centrifugation the haematocrit tube was cut above the blood cell phase and 8 μL of plasma was collected with a micropipette from the haematocrit tube. The micropipette containing the plasma sample was placed in a FluidX tube and immediately frozen at or below −20 °C. These shamtoxicokinetic samples were not analysed.

2.5. Terminal procedures

Animals were in their home cages with free access to food and water prior to termination on Day 15. They were not vehicle dosed on the morning of euthanasia. After the sham toxicokinetic 24 h blood sample (Groups 2 and 3) had been taken and terminal body