Regulatory Toxicology and Pharmacology 69 (2014) 425–433

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Assessment of haematological and clinical pathology effects of blood microsampling in suckling and weaned juvenile rats

Nicola Powles-Glover [a,*], Sarah Kirk [a], Lynne Jardine [b], Stephanie Clubb [b], Jane Stewart [a]

[a] Astrazeneca, Mereside, Alderley Park, Alderley Edge SK10 4TG, UK
[b] Charles River Laboratories, Tranent, Edinburgh EH33 2NE, UK

ARTICLE INFO

Article history:
Received 23 April 2014
Available online 19 May 2014

Keywords:
Microsampling
Pups
Juvenile
Weaned
Suckling
Haematology
Clinical chemistry
Clinical pathology

ABSTRACT

Tail vein microsampling in juvenile rats for toxicokinetic assessment has the potential to significantly reduce satellite animal use. This paper explores the toxicological consequences of microsampling at various post natal day (PND) ages.

Methods: Microsamples were taken as follows: suckling pups, 10 pups/sex, 3 × 32 μL samples on PND19, euthanased PND20; weaned pups, 10 pups/sex, 6 × 32 μL samples on PND23 and PND37, euthanased PND38; and satellite pups, 3 pups/sex, 5 × 32 μL samples on PND14 and PND35, euthanased on PND36. At termination on PND20 or PND38, clinical pathology samples were obtained and spleen, liver and bone marrow were examined. There were 10 unsampled concurrent control animals for each experiment.

Results: Suckling animals: females showed a slight, statistically significant decrease in red blood cell count (0.94× of control; $p < 0.05$) with slight decreases in haemoglobin and haematocrit. The suckling males showed a slight increase in reticulocyte counts (1.05× of control) plus a statistically significant, slight increase in relative splenic weight. Weanling animals: the only effect was decreased liver weight in the microsampled females. In both suckling and weanling experiments, all clinical pathology values were within the age control range. In the satellite pups microsampled on PND14, there was a statistically significant transient increase in bodyweight gain between PND17 and PND21.

Conclusion: The nature of the toxicological effects of microsampling was as expected. The magnitude of effects does not preclude microsampling main test pups provided care is taken over study design and blood volume loss.

1. Introduction

Non-clinical safety studies are sometimes required in juvenile rats to identify and characterise adverse effects and facilitate risk assessment for clinical studies in children. Toxicokinetic data are an essential component of non-clinical juvenile studies and are used to correlate circulating drug concentrations (exposure) with functional or pathological changes in the pups. Requirements for safety assessment and toxicokinetic evaluations 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 blood samples using satellite groups in rat studies has been identified as the largest influence on rodent numbers used in non-clinical safety studies (Sparrow et al., 2011). Satellite animals are routinely included on virtually all juvenile toxicity studies. In suckling rats this is often purely for collection of a single blood sample for toxicokinetic analysis using a terminal procedure. This means the number of satellite animals can often exceed the number of main test animals.

If an optimised microsample bioanalysis technique is available, a small volume of whole blood (32 μL) per time point is sufficient to measure plasma concentrations of circulating compound. Using a microsampling technique it is possible to remove multiple samples from adult rats at the start and end of a 14 day study without adverse toxicological consequences (Powles-Glover et al., 2014). These adult rat data have paved the way for adoption of microsampling of main test adult rats negating the need for satellite adult animals purely for toxicokinetic blood collection. Building toxicological profile pictures in this way allows for directly matching exposure data to clinical and pathological observations for individual animals (Chapman et al., 2014). As well as bringing the

* Corresponding author.
E-mail address: Nicola.powlesglover@astrazeneca.com (N. Powles-Glover).

http://dx.doi.org/10.1016/j.yrtph.2014.05.006
0273-2300/

scientific benefits linking exposure and toxicological information from the same pup, the use of capillary microsampling in suckling or weanling rats in juvenile toxicity studies has the potential to reduce the number of animals required per study even more dramatically than in adult animals.

Rat pups during the neonatal to weaning phase of development, i.e. from post natal days (PND) 1–21, typically range from 6 to 45 g in weight. Blood sampling can be technically challenging at these young ages. From a study design perspective, optimising sampling regimes is especially tricky in these younger animals because the blood loss from the sampling could potentially compromise the juvenile rat such that the toxicological profile of the test compound might be altered and/or clinical pathology observations could be rendered more difficult to interpret. In general, multiple (or even single) traditional volume (~200 μL) samples of blood for toxicokinetic purposes are not removed from main test pups (either suckling or newly weaned) on a regulatory juvenile toxicology study and satellite animals are used instead.

Systematic examination of toxicologically sensitive parameters in juvenile animals under controlled conditions has not been published previously to assess the toxicological, haematological and clinical chemistry consequences of removal of multiple microsamples at the start and/or end of short duration studies. The aim of this work was to investigate potential haematological, plasma chemistry (including the stress marker corticosterone) and organ effects, induced after in-life blood sampling for a sham toxicokinetic analysis in different ages of rat pups. Two experiments were performed either in the un-dosed suckling (Experiment A: PND19/composite profile – 3 time points, total volume 96 μL) or weaned (Experiment B: PND23 and 38/full profile – 6 time points each day, total volume 192 μL) juvenile rat. Spleen, liver and bone marrow were retained, weighed (except bone marrow), fixed, and processed for histological examination, no other tissues were retained. All terminal blood samples were evaluated for haematology, clinical chemistry and corticosterone. Assessment of clinical pathology parameters were carried out using terminal blood samples.

In addition, data are presented from a regulatory study in juvenile rats (Experiment C), where satellite pups were microsampled in vivo with a 5 × 32 μL regime (total 160 μL) on PND14 and PND35 and euthanased on PND36 after obtaining the last sample. The use of this method of blood microsampling allowed for in life sampling on the juvenile study where in previous designs a single large volume sample was taken as a terminal procedure from many satellite pups. Body weight and clinical observation data obtained from the microsampled satellite pups and from unsampled concurrent control pups are presented and discussed within the scope of this manuscript.

2. Material and methods

2.1. Animals, group size, housing and husbandry

2.1.1. Experiment A (suckling animals) and B (weaned animals)

Rcc™Han:WIST rats were used on this study. 80 Pups (40 males and 40 females) were divided into two blood microsampled groups and two age matched unsampled groups. Pups were randomised to groups based on litter of origin and individual bodyweights at the start of the experiment. Each group contained 10 animals per gender. A group size of ten was chosen to match the group size used on one month pivotal toxicity studies and typically used in juvenile rat studies.

Suckling animals were litter housed with the dam until weaning on PND21 when animals were multiple-housed in litter groups of up to 5 single sex animals/cage under standard conditions.

2.1.2. Experiment C (satellite animals)

Sprague–Dawley (Crl:CD(SD)) rats were used on this study. Pups were cross fostered from PND5–7 so that each cage consisted of 4 female and 4 male pups allocated to the same group but derived from different mothers to introduce genetic diversity. In the main test un-sampled control group there were 10 animals per sex, in the microsampled satellite group there were 3 animals per sex. Pups were dosed by oral gavage with purified deionised water at 5 mL/kg from PND14 to 35.

Pups were housed with the dam until weaning on PND21, when animals were multiple-housed in groups, 3 single sex animals/cage under standard conditions.

2.1.3. Housing and husbandry

Animals were kept under standard conditions e.g. in solid bottom, tinted plastic cages with woodchip bedding, room maintained at temperature 21 °C ± 2 and humidity 55% ± 15 on a 12 h light cycle. In all cages, water was 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 were provided for environmental enrichment.

2.2. Clinical observations and in-life monitoring

All pups were thoroughly examined after birth. 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 weight for all pups was recorded after birth and then daily to weaning and then twice weekly to termination. Food consumption was recorded for weaned animals twice weekly on the same days as bodyweight was recorded. At pup weaning on PND21, dams were killed and discarded without examination.

2.3. Tail vein microsampling for sham toxicokinetic analysis

For blood microsampled animals in Experiments A (suckling), B (weaned) and C (satellite), blood samples were collected using a toxicokinetic microsampling procedure (Jonsson et al., 2012). Briefly, 32 μL of whole blood was collected from the tail vein using haematocrit tubes (K_2EDTA treated) at each sampling time point. The haematocrit tube was plugged at one end with wax and stored on ice. Plasma was prepared and processed by a previously described method (Powles-Glover et al., 2014).

For Experiments A (suckling) and B (weanling), microsamples were taken from suckling animals (3 × 32μL samples per pup) on PND19 and weaned animals (6 × 32μL samples per pup) on PND23 and 37 according to a sham toxicokinetic blood sampling regime described in Table 1. A concurrent un-sampled vehicle control group for each age group was included in the study designs. Prior to in-life sampling all animals were gently warmed to approximately 38 °C in a hot box to encourage vasodilatation to aid procedural removal of venous blood from the tail vein. Time in the hot box differed depending on size/age of animal and sample size to be removed (data not shown). In brief, suckling animals that were microsampled on PND19 and weaned animals that were microsampled on PND23 were warmed for approximately 1–6 min prior to sampling. Weaned animals that were microsampled on PND37 were warmed for approximately 1 min prior to sampling.

For Experiment C satellite animals were blood microsampled on PND14 (suckling) and again on PND35 (weaned) collecting 5 × 32 μL samples on each sampling day. Animals were warmed in a hot box at 39 °C for between 5 and 10 min prior to sampling.