Clinical Chemistry Sampling and Assessment in Juvenile Rats – Reduction in and/or Elimination of the Need for Additional Subsets of Pups

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Abstract

Clinical chemistry parameters are routinely used in adult animals and are also used to assess function in juvenile animals. However, in juvenile animals there is a limit to the amount of blood that can be taken from each pup and to address these studies multiple subsets of additional animals with terminal endpoints are required. In contrast, microsampling has helped in the reduction of the volume of blood samples required for TK analysis in juvenile studies (Powles-Glover et al). To continue to reduce the number of animals needed for these studies, the sample volume required for clinical chemistry parameters was assessed but limited by the volume needed by the analyser for assessment and the survival of the assay in the sample is presented.

In view of this sufficient sample for a full clinical chemistry profile could not be obtained but a selection of key parameters was chosen to reflect clear changes over the lactation period (from neonate to weaning), assessed key target organs (heart, liver and kidneys) and were parameters that were suitable for quantification following dilution. Those selected were urea, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, globulin and AG ratio. Electrolytes were also analysed in non-dilute samples.

Samples were successfully collected from only 1-7 pups per group per sex (although more animals were sampled). The samples were collected from pups that had been dosed with water from post natal day (PND) 5. Samples of 0.15 mL were collected from the jugular vein on PND 14 and PND 21. Body weight for these animals was also assessed.

The results in a limited sample set demonstrate clear expected patterns over the time assessed. The numbers of animals used on juvenile studies can be reduced further if clinical pathology samples could be taken from main study animals via the jugular vein successfully, but further investigation of the sampling method and the effects on other toxicological endpoints would be needed.

Introduction

Juvenile study design has been a centre of focus over the past years with concern not only to produce a scientifically relevant study but one that also minimises animal usage. For toxicokinetic evaluation microsampling has been one of the key techniques successfully used to reduce the number of animals required to obtain full exposure data sets from pre-weaning pups, but sampling for clinical pathology at this early stage, although key in establishing target organ function at different developmental stages, would normally consist of terminal sampling in a number of rats. To reduce this usage and allow for animals assigned to this phase to be maintained on study we assessed a reduced volume sampling technique; a technique also being reviewed for use in adult toxicity studies.

Parameters were chosen based on previous in house work and work on performed on terminal neonatal samples (Papworth and Cludy, 1995). Parameters which had multiple isoenymes, showed little change with time across this developmental period or which could not be successfully diluted and measured were not evaluated.

Methods

As part of an ongoing investigatory study blood samples were taken via the jugular vein from pups on PNDs 14 and 21 for clinical chemistry assessment.

The samples were collected from pups that had been dosed with water from PND5 at a dose volume of 1ml/kg. The rat pups were held with one hand around the thorax, restraining the forelimbs and supporting the head; an appropriate sample for the analysis was then withdrawn by a Pasteur pipette. This was taken in a non-dilute sample set.

Samples that had been dosed with water from postnatal day 5 (PND 5). Samples of 0.15 mL were collected from the jugular vein on PND 14 and PND 21. Body weight for each pup was recorded daily. Body weight for these animals was also assessed.

The following methods were used.

Samples were run on an Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. Electrolyte levels were not measured at PND9 and 14; however, the greatest difference was expected were similar to those of an adult by PND 21, the limited number of samples on PND14 limiting the use of this parameter due to the sample volume required. The high urea level reflects a low osmotic pressure but also directly by decreased reabsorption in the neonate. Enzyme values are low in the immature liver. Insufficiency is attributed to the increase in enzyme levels and also in the size and function of the hepatoblasts to weaning.

For the lack of change in globulin levels the data are used cautiously.

Discussion

Sampling was easy and effective with a high rate of success. The jugular was a suitable sampling site and the analytical methods clearly detected changes across time. Clear changes in normal development of the liver and, to some extent the kidney and immune function, were seen and therefore any changes to this normal development pattern could be detected. The most suitable endpoints were urea, electrolytes, total protein and albumin – the lack of change in globulin highlighting the maternal preparation of the fetus and the addition of this element of the immune response post-natally. Electrolyte values as expected were similar to those of an adult by PND 21.