Effect of capillary microsampling on toxicological endpoints in juvenile rats at PND4, 10 and 17

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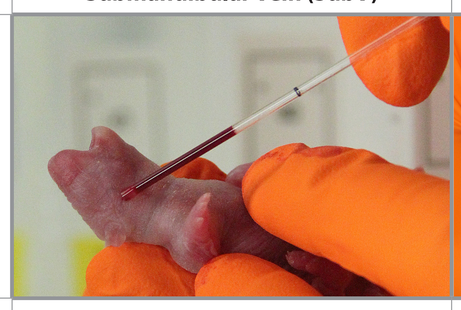
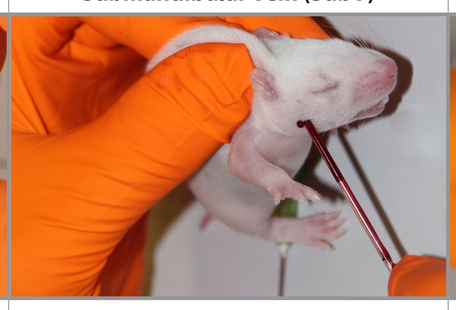
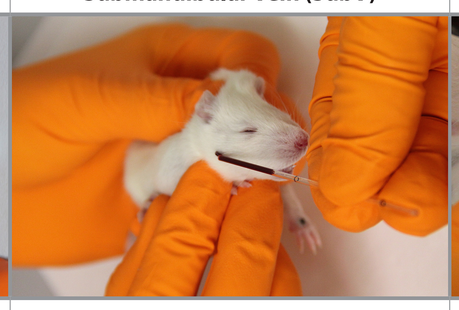
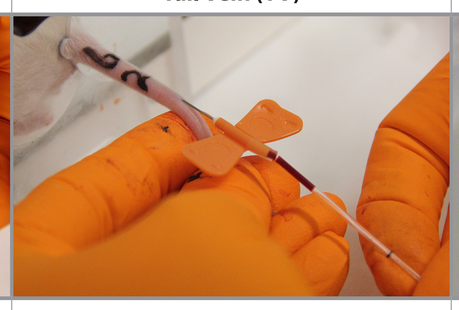
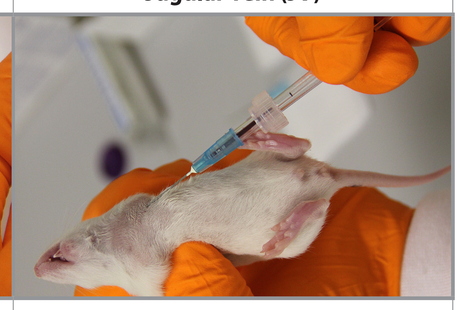
Introduction & Aim

- Non-clinical toxicity studies in juvenile rats are required by FDA and EMA, prior to performing clinical trials in pediatric populations. Toxicokinetic (TK) assessment of the tested compound is essential for each toxicology study.
- The conventional volume (200 µL) blood sample for TK assessment cannot be collected from main test pups, instead, satellite animals are used. Due to the small size of the juvenile rats, a terminal procedure is used to collect a single blood sample. A full pharmacokinetic profile requires repeated blood sampling, which means the number of satellite animals can often exceed the number of main test animals.
- Capillary Microsampling (CMS) is a technique for obtaining small amount of blood (~30 µL/ time point). This technique makes it possible to collect multiple blood samples from one rat pup, thereby reducing animal use in TK assessments.
- We have proved that CMS is feasible to collect multiple blood samples from juvenile rats at 4, 10, and 17 days of age. One remaining question is whether CMS would compromise key toxicological endpoints.
- In this project, we investigated the effects of CMS on toxicological endpoints including body and organ weights, clinical laboratory parameters and clinical signs.

Materials, Methods & Results

Wistar Han juvenile rats at postnatal day (PND) 4, 10 and 17 underwent CMS via submandibular vein (SubV), tail vein (TV) or jugular vein (JV) for a maximum of 3 times in a 24-hour interval. Animals were terminated one day (acute group) or 7 days (chronic group) after CMS; blood was collected at termination for clinical laboratory parameters by decapitation or from the aorta when deeply anaesthetized using isoflurane.

Acute and chronic effects of CMS on haematology and bio-chemistry parameters, body and organ weights (spleen and liver, only chronic) were tested. Data were compared between CMS groups and their concurrent un-sampled controls. Clinical signs were monitored in life. The study design and summarized results are shown in the table below.

	PND4 (5/sex/group)	**PND10 (5/sex/group)**	**PND17 (5/sex/group)**		
Method	**Submandibular Vein (SubV)**	**Submandibular Vein (SubV)**	**Submandibular Vein (SubV)**	**Tail Vein (TV)**	**Jugular vein (JV)**
Photo					
Frequency	3 x 32µl* 2 x 32µl* 1 x 32µl	3 x 32µl*	3 x 32µl*	3 x 32µl	3 x 32µl
Time Points (Hrs Post Dose#)	0.5, 2, 4 0.5, 2 0.5	0.5, 2, 4	0.5, 2, 4	0.5, 2, 4	0.5, 2, 4
Termination	PND 5 (Acute) PND 11 (Chronic)	PND 11 (Acute) PND 17 (Chronic)	PND 18 (Acute) PND 24 (Chronic)	PND 18 (Acute) PND 24 (Chronic)	PND 18 (Acute) PND 24 (Chronic)
Clinical signs	All pups: a bruise on cheek after one time sampling; haematomas disappeared the day after	No findings	3/20 pups: clonic spasms and abnormal posture during blood sampling; animals terminated immediately after sampling	No findings	No findings
Body Weight	No difference	No difference	No difference	No difference	No difference
Organ Weight (Spleen and Liver)	No difference	No difference	No difference	No difference	No difference
Haematology	**Acute group males and females:** NA **Chronic group males:** 3 x SubV group: white blood cells ↓ **Chronic group females:** 3 x SubV group: haemoglobin ↓	**Acute group males and females:** Red blood cells, haemoglobin, haematocrit ↓ Monocytes, reticulocytes ↑ **Chronic group males and females:** Mean corpuscular haemoglobin concentration (MCHC) ↓	**Acute group males:** No difference **Acute group females:** Red blood cells, haemoglobin, haematocrit, MCHC, neutrophils ↓ **Chronic group males and females:** No difference	**Acute group males:** Red blood cells ↓ **Acute group females:** MCHC ↓ **Chronic group males:** Monocytes ↓ **Chronic group females:** No difference	**Acute group males:** Red blood cells ↓ **Acute group females:** MCHC ↓ **Chronic group males and females:** No difference
Bio-Chemistry	**Acute group males and females:** NA **Chronic group males and females:** No difference	**Acute group males:** Triglycerides ↑ **Acute group females:** No difference **Chronic group males:** Glucose ↑ **Chronic group females:** No difference	**Acute group males:** No difference. **Acute group females:** Triglycerides ↓ **Chronic group males:** Alanine aminotransferase (ALAT) ↑ **Chronic group females:** No difference	**Acute group males:** No difference. **Acute group females:** Triglycerides ↓ **Chronic group males and females:** No difference	**Acute group males:** No difference. **Acute group females:** Triglycerides ↓ **Chronic group males and females:** No difference

* Repeated sampling is possible by alternating the side of cheek.
# Animals were not actually dosed and so "time after dose" refers to time after 8 a.m. which would be a typical time of dosing in a once daily dosing study.
NA: not applicable due to insufficient blood volume for these assessments on PND 5.
No difference: no difference compared to the control group.
↑: increase ↓: decrease

Summary & Conclusion

- Removing maximum 3x32 µl blood over a 24 h period did not adversely affect standard toxicological endpoints such as clinical observations, body weights, organ weights or biochemistry parameters in both male and female pups which were microsampled at PND 4, 10 and 17.
- Acute effects of CMS on haematology parameters were found. Changes such as decreased red blood cells, haemoglobin and haematocrit, and increased reticulocytes were observed. Among those, decreased red blood cell count, which appeared in all the acute groups, can serve as a sensitive biomarker for the CMS induced acute blood loss.
- Most of the changed haemotology parameters came back to control levels after 7 days, indicating a good recovery of the animals. Decreased haemoglobin remained in the PND4 chronic group when the pups were microsampled for 3 times, suggesting a maximum of 2 CMS samples in 24 hours for PND4 pups. As acute effects on haematology parameters were observed, it is not suggested to use the main animals for TK blood sampling.
- CMS is feasible in juvenile rats for TK assessment. Utilizing this method could reduce the use of satellite animals 3–6 fold.

EUROTOX 2015

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