Microsampling – A Toxicologists Perspective

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GSK
3Rs

• Reduction
• Refinement
• Replacement
Why am I here?
Why is Microsampling so important?

You want how much?

How much do you need?

How much have you got?

5.6ml!
Blood Volume Limits - Rats

15% CBV (mL) in 4 weeks

Body Weight (g)

Blood Volume (mL)
Delivering the Science but committing to 3Rs
Relating microsampling to circulating blood volumes *

- Typical guidance:
  - no more than 15% of circulating blood volume taken in a 28 day period

- In a 250 g rat, having a circulating blood volume of 16 mL, 2 x 8 point profiles using 50 µL microsamples requires 0.8 ml blood which represents around 0.5% of total blood volume.

- This may be compared to the commonly used 2 mL blood withdrawal from an 8 kg dog which corresponds to approximately 0.3%.

*Diehl et al. A good practical guide to the administration of substances and removal of blood, including routes and volumes. J. Appl. Toxicol. 21, 15-23 (2001)
Blood Volume Limits - Rats

15% CBV (mL) in 4 weeks
Potential benefits of microsampling

- **Refined blood sampling procedures:**
  - reduced blood loss, alternative sampling sites, quicker and less stressful sampling (e.g., warming)

- **Reduced number of animals:**
  - TK (and MIST) samples from main study animals

- **Scientific gain:**
  - Relate toxic effects to exposure in the same animals

- **Business benefits:**
  - Reduced compound requirements (particularly valuable in early studies)
  - Reduced resource (no dosing, housing, care required for additional satellite animals)
So what’s stopping us?
Investigations into Consequences of in-Life Sampling in Rats
Retrospective Data Analysis

- Six studies where Control animals were not sampled during the in-life phase

- Six studies where Control animals were sampled up to 6 times over a 24-hour period (approximately 200 to 350 μL blood per sample) on days 7/8

- Looked at control animal data only, N=4 Males per study
  - Clinical Pathology Standard profile
    - Plus reticulocyte sub populations
  - Pathology Standard list
Haematology Data

%H change from 'Not sampled' to 'Sampled' data

% Change

Red cell decreases

Absolute reticulocyte count not changed

High absorption reticulocyte population increased (x1.83)

No Pathology changes
Summary of retrospective data analyses

- Close examination of reticulocytes sub-populations gives indication of reticulocyte activity before there is a significant change in the reticulocyte total count.
- This comparison was with healthy control animals - males only.
- Animals killed immediately after TK bleed so no compensatory effect could be observed.
- What effect would we see if the animals were compromised?
- What about females?
- What would happen if two TK profiles were collected, – Days 1 and 7?
Investigative Study

- To assess any in vivo consequences of blood sampling in SD rats
  - Male and female treated and control animals
    - To assess any difference in physiologically impaired animals compared with control
    - Exacerbation of compound induced effects?
    - Gender differences (females are smaller)?
    - Sample volume impact?
  - Acetaminophen (APAP) at 600 mg/kg/day
  - 7-day dosing duration

“All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.”
Parameters Measured

- Standard Haematology plus reticulocyte sub populations
- Standard Clinical Chemistry
- Additional parameters considered to be stress indicators
  - ACTH
  - Corticosterone
- Pathology
  - Spleen (site of red blood cell production)
  - Thymus or thymic area (changes often associated with stress)
  - Sternum with bone marrow (site of red blood cell production)
<table>
<thead>
<tr>
<th>Dose APAP* (mg/kg/day)</th>
<th>Dosing Duration (Days)</th>
<th>TK Day 1</th>
<th>TK Day 7</th>
<th>Approx Sample Vol (μL)</th>
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*4/sex/group

**Non sampled controls**

**Are there any compensatory changes 7 days after TK sampling?**

**Are there changes comparable to those seen in the retrospective data analysis (previous sample vol 200-350μL)?**

**What the effects of microsampling on Days 1 and 7?**

**Are the effects reduced with a smaller blood volume?**
Investigative Study Design

Non sampled controls

Are any changes comparable to those seen in the retrospective data analysis (previous sample vol 200-350μL)

Are there any compensatory changes 7 days after TK sampling?

What the effects of microsampling on Days 1 and 7?

Are the effects reduced with a smaller blood volume?

**Majority of sampled Groups**

- Slight effect in Spleen
- No changes in Thymus (sampling-related)
- No changes in Sternum bone marrow

- Small decreases in red cell parameters
- Small increases in total reticulocytes
  - Primarily % high absorption population

- Incidence and severity of spleen changes increased with both Day 1 and 7 sampling in females only
- Magnitude of haematology changes increased with both Day 1 and 7 sampling in both sexes
- Magnitude of changes decreased with smaller sample volume
Conclusions

• Changes in reticulocyte sub-populations can occur following blood sampling even in the absence of changes in total absolute counts

• High absorbance/fluorescence reticulocytes are a good reliable early marker of changes

• TK blood can be taken from Tox animals using microsampling without adversely affecting standard parameters

• Reducing sample volume may further reduce any effect
Considerations for Removal of TK Satellites

• These data formed the basis for proposing the removal of satellite rats from non-GLP toxicity studies.
• There will be changes in toxicology animals when used for TK.
• This should not affect overt toxicity assessment, e.g., hepatotoxicity, renal toxicity.
• HOWEVER, collection of TK samples from toxicology animals may be inadvisable with some compounds:
  – Bone marrow is target organ.
  – Haematopoietic tissue is target.
  – Haemodynamic effects anticipated.
  – Haemotoxicity.
• Not ready to change on GLP studies at this stage.
Was Dried Blood Spots (DBS) the answer?

- Began investigating use of DBS within GSK in late 2006
- Pilot *in vivo* WW-Safety Assessment studies started in January 2007
- World-wide agreement and implementation of DBS in January 2008, where appropriate
  - Preferred technique for assessment of TK of compounds selected as candidates
  - Would progress into clinic
- No regulatory reason not to use blood

- Job done......
The ups and downs of DBS....
The ups and downs of DBS....

- Understanding of the technique is continually evolving
  - Learning where the technique *is* and *is not* suitable, as well as potential hurdles
  - More difficult for bioanalytical group; advantages are to preclinical and clinical groups
    - ‘Validation’ is more extensive than for a plasma based assay

- Regulatory agencies are taking conservative approach to its use
  - For the time being, we are expected to employ sparse sampling approach of wet blood to compliment DBS samples and show continued concordance

- One of the biggest concerns with the technology is the hematocrit effect
How is Haematocrit (Hct) affected?

- **Dehydration** – less plasma so Hct goes up
- **Chronic haemorrhage (blood loss)** – less blood cells in circulation so Hct goes down
  - Especially from GI tract
- **Bone marrow toxicity** – destruction of RBC’s progenitors leads to decreased Hct
  - Oncology agents can produce this effect
- **Iron deficiency** – lower Hct
- **Chronic kidney disease** – lack of production leads to decreased Hct
- **Test articles** can have primary effects (increased or decreased) on Hct based on mechanism of action (e.g., increased production of RBC’s) or secondary effects (e.g., dehydration)
- **Wide variation in haematocrit between species and with age in humans**
How do changes in Hct affect DBS?

• When haematocrit (Hct) is high there tends to be less spread of the spot. The higher percentage of red blood cells (RBC’s) in the blood. This can have the effect of lower extraction meaning less compound can be extracted from the dried sample.

Fig 2

- Spot 1 – normal range Hct
- Spot 2 – mid/high Hct
- Spot 3 – High Hct
Impact of haematocrit effect
Continuing along the microsampling road
Moving the road block?

• Collecting the whole blood spot (not a punch from the middle of the spot) could also alleviate this Hct bias?
  – Relies on the volume being accurately collected and spotted – may be issues in clinical situations?
• Partial sampling methods introduce unwanted bias owing to inconsistent spot sizes and possibly non-homogeneous spots due to chromatographic effect
• Whole spot methods eliminate the variation from spreading and non-homogeneity
• They allow for more consistent DBS concentrations, even at different haematocrit levels

• and.....
Currently 3 x 15ul spots

- Requires 5mm punch from centre of spot
- Allowance for volume inaccuracy as accuracy from analyst punching spot

Volume reduction

Proposed 3 x 5ul s

- Entire spot taken by analyst
- Spot volume must be accurate
- Potential for inaccuracy as no visual cue to see differences in spot size
- No allowance for inaccuracy
Finding an alternative route?

Plasma Microsampling?
Plasma Microsampling?

- Allows use of small samples
- Eliminates Haematocrit issues
- Addresses conservative approach
  - It's still plasma!
- Practicalities
  - Creative thinking....
Overcoming Technical Hurdles

Sample tubes

Centrifuges

Capillaries
Conclusions
Realising the Benefits

- Reduce, refine, replace
  - Satellite animals have been removed from many non-pivotal studies.
  - Sampling from main study animals on pivotal studies needs to follow...

  - Use of a composite design may be the way forward?
Default TK design for 1 or 3 month study, all main study animals sampled

A balanced study design with 10 animals, composite TK with 6 time points and 3 samples per animal, 5 samples at all time points, 30 TK samples per group in total (previously 18 per group in total using satellite animals and serial sampling)

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Other potential microsampling benefits

• Most significant 3Rs gain is reduction in rodents
  – Juvenile rats
  – Mice
  – Smaller rats (HW vs SD)
• Decreased sample volume in monkeys – refinement
  – Multiple analytes so volume is key, particularly for biopharm
• Opens options for different sampling methods - refinement
  – Eg. Ear vein sampling in dog
  – Less invasive methods
• Ability to sample other fluids where volume if limiting
  – CSF ?
  – Mouse urine ?
• Non-drug analytes
  – Explore for clinical pathology?
• Sampling from seriously ill patients and small children
Questions?