Introduction

In drug development projects, compounds with promising in-vitro properties are selected for in-vivo testing to look at basic properties such as tolerability and blood levels. Many compounds are eliminated from screening cascades at this step as they do not have the appropriate drug-like properties to produce positive results in later disease models.

In the majority of cancer drug discovery projects this initial in-vivo work tends to comprise of a rapid test to make sure there is no overt toxicity that would cause animal welfare issues (known as a tolerated dose study or TD) followed by a pharmacokinetic study (or PK) to investigate drug absorption and elimination.

Using mice in routine PK studies can be challenging as the total blood volume of mice, often less than 1.5 ml, limits the size and number of samples that can be collected. Advances in bioanalytical techniques have opened up the potential to use smaller sample volumes (microsamples) to assess drug exposure, whilst minimising the physiological effect of taking large blood volumes.

Historically within AstraZeneca Oncology groups we have used two mice on the TD study and a further ten mice used to provide historical tolerated dose (2/3 mice) to obtain more dynamic PK measurements.

In addition we have introduced serial bleeding taking samples during the time course of chronic studies (tolerance and efficacy) to obtain more dynamic PK measurements.

Method

Adequate training is necessary for blood collection using the microsampling technique. This bleeding technique can be followed after warming the mice in a warming chamber to dilate the blood vessels prior to taking the sample (however more experienced individuals find this warming step is not necessary).

Alternate sides of the tail should be used and successive needle punctures moved towards the tail base.

Samples are collected by a simple prick to the tip of the mouse's tail with a 25g needle. The blood is collected accurately with an EDTA coated 20ul micro capillary tube.

Samples are centrifuged to give a diluted plasma supernatant.

The blood sample is diluted into 80ul of PBS

The supernatant is removed (75ul) and frozen in a 96 well plate. These samples are now ready for analysis.

Once the sample has been taken blood flow is stopped by applying finger pressure on a piece of soft sterile swab placed at the blood sampling site before the animal is returned to its cage.

Advantages of Microsampling

There are multiple advantages associated with microsampling including scientific, resource related and animal welfare benefits.

- Smaller sample volume
- Less trauma
- Less recovery time
- Reduced housing space
- Improved accuracy
- Reduced costs
- Improved sensitivity

Validation Data

Before this method was routinely adopted by the Oncology group at AstraZeneca, Alderley Park there was a significant amount of data generated comparing composite PK studies (cardiac puncture or vena cava) and tail vein microsampling (serial) to ensure that the data was comparable between the two methods.

Examples of the data sets shown below demonstrate the accuracy and scientific validity of using this technique for PK analysis.

Conclusions:

The utility of capillary microsampling within in-vivo research is becoming evident as more researchers demonstrate that this is a valuable method for collecting serial samples from mice and rats.

The methodology has been adopted globally with in AstraZeneca and we have influenced a number of CROs to use our method for plasma PK microsampling.

More dynamic PK measurements from studies are possible eg, d1, d7, d14, d21 samples from the same animal to measure exposures over time.

Significant reduction in number of animals used for Oncology in-vivo studies at AstraZeneca.

Future Improvements:

Further improvement of LCMS sensitivity for PK analysis may reduce blood volumes required further.

Improved sensitivity will allow analysis of platinum agents in the blood – not currently possible with our method.

Extend microsampling technique to investigate haematological, cytokine & immune –phenotype analysis of blood samples.

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