Investigation of micro blood sampling from the marginal ear vein of the Göttingen mini-pig for non-clinical safety studies.

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Abstract

Over recent years, the use of the mini-pig for non-clinical toxicology studies has expanded, leading to the species becoming a viable non-rodent alternative, accepted by regulatory authorities. Although the mini-pig is a useful model due to possible extrapolation to man, the increased demands for blood sampling in non-clinical studies is considered a drawback. Traditional blood sampling routes, such as the cranial vena cava, can result in excessive stress over repeat occasions, and although alternatives exist (e.g. vascular access ports), surgery is typically required, highlighting the need for an alternative approach.

In this study, we have demonstrated the ability to collect blood samples from the mini-pig on repeat occasions, using a microsampling technique. Blood samples were collected from the marginal ear vein, by capillary action after venepuncture. Micro blood samples (approximately 40 µL) were obtained on six occasions on Day 1, with the procedure repeated the following week (Day 8).

Samples were collected successfully, with a notable reduction in distress to the animals. Slight bruising to the ear was noted, but recovered rapidly and did not impair further sample collection.

For future studies, this approach would be considered suitable for obtaining repeat blood samples from the marginal ear vein of the mini-pig, with the added benefit of alleviating stress compared with existing techniques.

Introduction

Since the Göttingen mini-pig was first introduced to the field of safety assessment in the late 1960s, its acceptance as a viable alternative non-rodent species has grown exponentially. From its selection for dermal research when first introduced, to its wide spread use in toxicology and pharmacology studies today, the species' acceptance by regulatory authorities is clear and the strong correlations of the mini-pig's anatomy, physiology and biochemistry with man continue to demonstrate its potential for further development.

Despite the diverse advantages associated with the species, one drawback remains relating to the ability to obtain serial blood samples. Typically, blood samples are withdrawn from the cranial vena cava, as this is one of the most readily accessible veins for the withdrawal of large volumes of blood on a single occasion. However, serial sampling from the cranial vena cava over a short period of time can cause stress, leading to animal welfare concerns and potential non-test-item-related mortality. Furthermore, although surgically based blood sampling approaches have been developed, for example vascular access ports, surgical preparation of animals should be avoided, where possible, for regulatory toxicity studies.

One alternative non-surgical blood sampling route for the mini-pig has evolved with the development of micro-blood sampling techniques, such as whole blood, serum or plasma capillary sampling or the dried blood spot technique (DBS). Since these approaches require minimal volumes of blood (typically less than 50 µL), routes for sampling once thought to be unsuitable can be revisited. In the case of the mini-pig, the marginal ear vein is the most readily accessible option for blood sample collection, but was previously thought to be unsuitable due to the tendency of the veins to collapse when withdrawing large volumes of blood, potentially preventing access to them for the purposes of euthanasia. The micro-blood sampling approach holds the potential to overcome these barriers.

At Sequani, the ability to collect blood samples from the marginal ear veins of the mini-pig using a micro-sampling technique was evaluated. This poster provides details on the conduct of the procedure and how this technique can be readily applied as a non-surgical alternative for blood sample collection on regulatory non-clinical toxicity studies in the mini-pig.

Methodology:

Study Design:

The study was designed to allow for two distinct phases:

Phase 1 - To assess the suitability of blood sample collection from the marginal ear vein at a single time point using the micro-sampling approach (first sample on Day 1).

Phase 2 - To assess the ability to collect blood samples at multiple time points for a toxicokinetic profile, as may be required in the context of a non-clinical toxicology study. Blood samples were collected at six time-points on Day 1, with a further six time-points one week later (on Day 8). The sampling times were selected to mimic those frequently selected in a non-clinical toxicology study (0 [1st sample collection], 1, 2, 4, 6 and 24 hours).

Only once the blood sampling route was confirmed as suitable in Phase 1, did the study progress to Phase 2.

Animals:

Ten male and nine female Göttingen mini-pigs (supplied by Ellegaard, Denmark), approximately 7 to 8 months old, were allocated to the study. The animals were housed in groups of two or three, and had been acclimatised to their environment for approximately 6 weeks before the first blood samples were collected.

Blood sampling procedure:

Since an intact marginal ear vein is required for the euthanasia of mini-pigs, the number of attempts to obtain a blood sample was limited to three per blood sampling occasion. Aseptic conditions were also implemented to prevent possible contamination and infection, and a local anaesthetic cream was applied to the ears, where considered appropriate, to reduce discomfort (used at the 6 hour blood sampling time point on Day 1 for the females and for all animals at the 24 hour point on Day 1 and all samples on Day 8).

To prepare the animals for the procedure, the peripheral ear veins were dilated by applying a swab soaked in warm water and gently stroking the area to promote vasodilation. The vein was also occluded at the base of the ear using an elastic band, which was kept in place throughout the procedure. Attempts to use an alcohol swab or Vasolate (40% d-limonene) to dilate the veins were also made, but the effects of alcohol evaporation cooled the surface of the ear, thereby reducing blood flow. Animals were restrained manually.

Mini-pig restraint for the blood sampling procedure and visualisation of the marginal ear vein.

Needle position for venepuncture of the mini-pig marginal ear vein.

When collecting blood, the needle was inserted in the marginal ear vein, with the bevel of the needle orientated towards the base of the ear, alternating between the two ears for each time-point. Twenty-one or 23 gauge needles were used, with the choice of size made at the discretion of the technician, based on the size of the animal. Blood was collected from either the hub of the needle in situ or by collection from the surface of the ear after venepuncture. A 40 µL end-to-end capillary tube containing EDTA as anticoagulant and manufactured by Sarstedt Microvette® 100 was held against the blood, to collect a sample by capillary action.

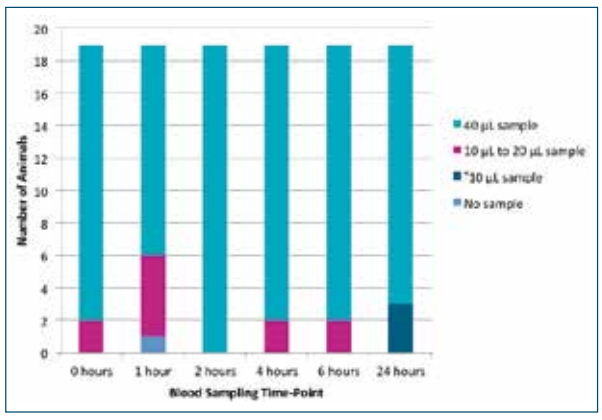

Figure 1: Number of blood samples and sample volume at each time point on Day 1.

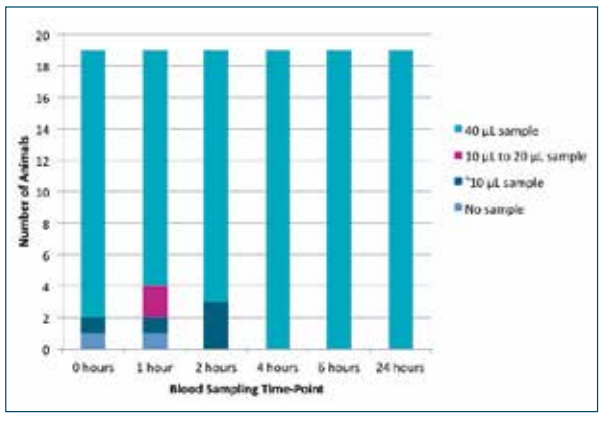

Figure 2: Number of blood samples and sample volume at each time point on Day 8.

Discussion / Conclusion

This study successfully demonstrated the suitability of blood sample collection from the marginal ear veins of the Göttingen mini-pig, using the micro-blood sampling approach. Not only was it possible to collect blood samples of required volume (40 µL), on the majority of occasions, but minimal distress was induced by the procedure. The integrity of the marginal ear vein was also maintained, with only a small needle puncture and some slight bruising to the ears which rapidly regressed. The success in avoiding damage to the marginal ear vein was attributed to sampling as close as possible to the distal end of the ear and by good animal restraint; restraint of the animal over the handler's lap, covered with a vet bed, was considered the most suitable option.

There were occasions when it was not always possible to obtain a blood sample, or that the volume collected may have been inadequate for bioanalysis (10 µL samples). However, these instances rarely affected the same animals twice, suggesting that individual systemic exposure information could still be derived despite these occasional shortfalls. Overall, it was concluded that this technique was successful and would be considered suitable for obtaining repeat blood samples from the marginal ear vein of the mini-pig for non-clinical toxicity studies, with the added benefit of alleviating stress compared with existing techniques.

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