

Central Venous Catheterisation of the Göttingen Minipig by Jugular Vein Placement

An Investigation of Potential Utility in Regulatory Safety-Evaluation Studies

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Introduction

In recent years, the use of the minipig for non-clinical toxicology studies has increased due to the model's anatomical, physiological and biochemical dispositions that allow extrapolation to humans. This has resulted in the minipig becoming a viable non-rodent alternative that is accepted by regulatory authorities. Furthermore, as a wealth of background data accumulates and ethical concerns continue to be raised by the public over the use of other non-rodent species such as the dog, there seem to be few factors that could limit the minipig's potential to become the primary non-rodent option.

One drawback of minipig use for non-clinical toxicology studies relates to the obtaining of serial blood samples. Usually, blood samples are obtained from the cranial vena cava due to the vein's easy access and the fact that large blood samples can be taken on a single occasion. However, with the increasing complexity of non-clinical studies, the need for more extensive blood sampling is apparent.

Serial sampling from the vena cava over a short period of time can cause stress in the minipig, leading to animal welfare concerns and potential non-test-item-related mortality. This has required the development of surgically-based blood-sampling approaches, such as the installation of vascular access ports and, whilst this approach is certainly appropriate for some

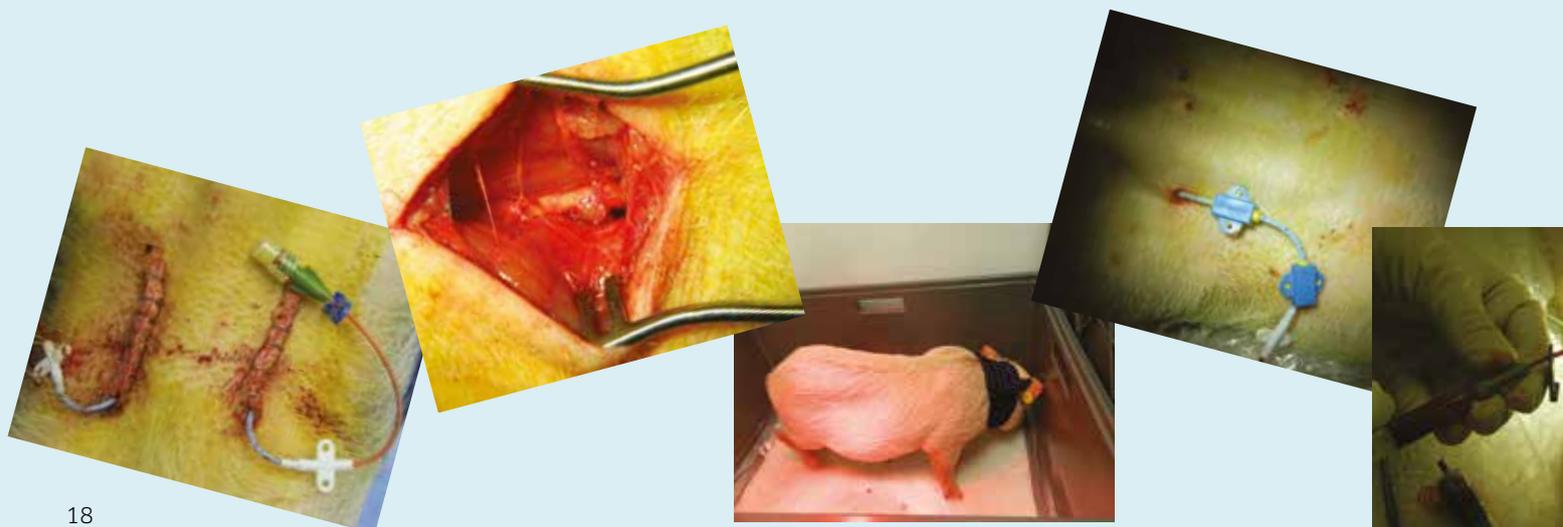
study types, it is not ideal for use in routine regulatory toxicity studies. A positive advancement in this area appears to be the previously described method for the minimally invasive insertion of a central venous catheter into the external jugular vein, allowing the facilitation of multiple blood samples.

At Sequani, we have investigated the placement of a catheter in the external jugular vein. The study design is briefly discussed in detail, yet with the extensive technical details available in Ellegaard's catheter implantation educational package, the aim of this article is to share our first-hand experience with the technique and its potential use in future regulatory studies.

Study Design

The primary objective was to investigate the practicality of central venous catheterisation of the jugular vein, for potential use in regulatory non-clinical studies. Six minipigs were divided into three groups with the intention to study the insertion of a catheter for a period of time that would be useful in obtaining the toxicokinetic samples required in a one-month regulatory study.

On each occasion, a catheter (Arrow Gauge 14) was inserted using the Seldinger technique with the animal anaesthetised. The catheter was removed the next day following serial blood sampling for two groups, and the catheter remained in-situ for



an additional group to assess patency. Patency was assessed by visible signs of infection at the insertion site and by means leucocyte parameters analysed from the blood samples obtained. During the final catheterisation, a cut-down technique was employed to visualise the veins during the procedure. Blood-sample quality and the ability to reinsert the catheter were also assessed.

Discussion

Catheterisation Practicability and Success

Insertion of a catheter into the jugular vein became increasingly successful as the technicians gained first-hand experience and became accustomed to the procedure.

A total of 17 catheterisations were attempted (not including occasions where a cut-down method was applied); 11 were successful resulting in a catheterisation rate of 65%. The low success rate was a result of the first three catheterisations where none was successful. After these occasions where procedural experience was low, the catheterisation rate improved to 79%. The burden of the remaining unsuccessful catheterisations involved two animals where the first catheterisation attempt was unsuccessful and future attempts were made within a seven-day period. Surrounding vessels had been punctured causing swelling at the injection site, and insufficient recovery time was the likely cause of these unsuccessful attempts. It was found that animals could be successfully catheterised following a 14-day recovery period.

Experience in catheter insertion was associated with a clear reduction in the time taken to conduct each procedure, with later catheterisations being around 60% faster than the initial ones.

Patency and Re-insertion of Catheters

Patency was demonstrated for a period up to 22 days with no changes in related blood parameters or signs of infection. Longer patency is possible but could not be further demonstrated in this study due to one catheter becoming dislodged and another being damaged and having to be replaced.

Self-inflicted removal of the catheter by one minipig resulted in no injury to the animal and future removal was prevented by the adaptation of a neck collar to secure the catheter. This

unintended removal allowed us to demonstrate the ability to reinsert the catheter on the same day or the day after removal. Reinsertion potential was also demonstrated on fortnightly occasions over a one-month period.

Blood Sample Quality

Blood sample quality was unaffected, with haematology and blood chemistry parameters comparable to background data collated from other collection routes. A number of dubious values was seen for standard coagulation parameters; however, this was attributed to heparin contamination from incomplete flushing of lines. This was resolved by more thorough flushing prior to blood sample-taking and resulted in coagulation parameters within the expected range.

Cut-down Method

On all occasions where the cut-down method was used, the insertion of the catheter was successful. Procedural times were longer than those where only the Seldinger technique was used; however, visual access to the vein resulted in easier catheterisation and allowed the insertion of a catheter into both the left and right veins simultaneously.

Anaesthetic Regime

Minipigs were anaesthetised using a regime of injectable anaesthetics/analgesics comprising ketamine, medetomidine hydrochloride and butorphanol, as well as isoflurane through a nose cone. The amounts of injectables were reduced for each catheterisation to investigate the minimum required for a regulatory non-clinical study without compromising the state of anaesthesia. Initially, vocalisation and agitation were observed in one animal for the administration of ketamine; this was subsequently removed from the regime with no adverse effects on the state of anaesthesia. Reducing the remaining injectable anaesthetics shortened the recovery time following the procedure and subsequently reduced the level of agitation in the animals as they regained consciousness faster.

Conclusion

Central venous catheterisation of the jugular vein appears to be a promising alternative to serial blood sampling in toxicology studies involving the minipig. With experience, the insertion of a catheter becomes relatively quick and subsequently reduces



the manpower required for blood sampling. More importantly, a large reduction in stress was apparent which improved the welfare of the animal. Blood samples were obtained with ease on all occasions with the offering of a food treat eliminating the need for a sling or excessive handling.

These preliminary investigations suggest that the technique could be used in regulatory toxicology studies incorporating toxicokinetic bleeds that require serial blood samples. There are some issues concerning the amount of time needed to conduct the procedure in a study that may include 32 animals or more. Nevertheless, with well established procedures in place, this could easily offset the work involved in repeated blood sampling using the traditional method. At present it would seem appropriate to insert catheters for short periods to cover each serial blood-sampling session only (usually at the beginning and end of the study). However, further investigative work into line patency could open up the option of keeping catheters in place for a full one-month regulatory study.

The appeal of this technique would be further enhanced by adapting the anaesthetic regime to solely involve the administration of isoflurane through a nose cone, i.e. without any

injectable anaesthetics. This requires further investigation but could substantially accelerate the recovery time.

In conclusion, we intend to further develop and enhance the procedure before introducing it into a regulatory study. However the progress made in this initial investigation will enable us to conduct additional small-scale studies using the technique and thereby develop proficiency and process efficiency at a suitable level. It should be noted that where less extensive blood sampling is required, bleeding from the cranial vena cava using the traditional method would still be considered the most appropriate.

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