



Technical Report

PET radioligand injection for pig neuroimaging

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Summary

Pigs are useful models in neuroimaging studies with positron emission tomography (PET). Radiolabeled ligands are injected intravenously at the start of the scan and in pigs the most easily accessible route of administration is the ear vein. However, in brain studies the short distance between the brain and ear vein can be problematic as both are localized inside the field of view and, as a consequence, tracer residues in the catheter may influence the outcome of the scan. Here, we discuss options to avoid this problem. The femoral vein can be used in studies where repeated arterial blood sampling is needed because surgical incision has to be performed to allow access to the artery. When a non-invasive technique is preferred, the ear vein is a good alternative although it is recommended to dilute the tracer sufficiently in saline (20-50 mL) prior to injection. In addition, the tracer can be injected through an extension tube (filled with saline before injection), which is removed together with the syringe immediately after tracer injection. This avoids placing the syringe with tracer inside the PET gantry while injecting. By applying these simple techniques, it is our experience that it is possible to obtain high-quality images without exposing pigs to invasive procedures.

Introduction

Positron emission tomography (PET) is a technique for the study of physiological and pathophysiological processes in the living organism (Bailey *et al.*, 2005). Along with other imaging modalities, PET is a useful tool to gain insight into the *in vivo* functions of the healthy and diseased brain. For this purpose, the pig is a suitable animal model as it has a relatively large gyrencephalic brain with high resemblance to the human brain, which is an advantage due to the low spatial resolution (3-5 mm FWHM) with PET (Alstrup & Smith, 2012). Furthermore, the translational value is strengthened by use of human scanners. In PET, a tracer is administered intravenously, after which the scanner measures the emitted

photons and images of the tracer distribution in the brain can be reconstructed (Bailey *et al.*, 2005). The vein that superficially gives the easiest accessibility in both minipigs and domestic pigs is the ear vein, and it is generally used in pre-clinical studies (Alstrup, 2010; Bollen *et al.*, 2010; Gillings *et al.*, 2001; Landau *et al.*, 2017). However, in brain studies the short distance between the brain and ear vein can be problematic, as both are localized inside the field of view, and as a consequence tracer residues in the catheter may influence the outcome of the scan. Although only a very small volume of the tracer adheres to the catheter, such a highly concentrated point source can affect the quality of the image as it increases the inci-

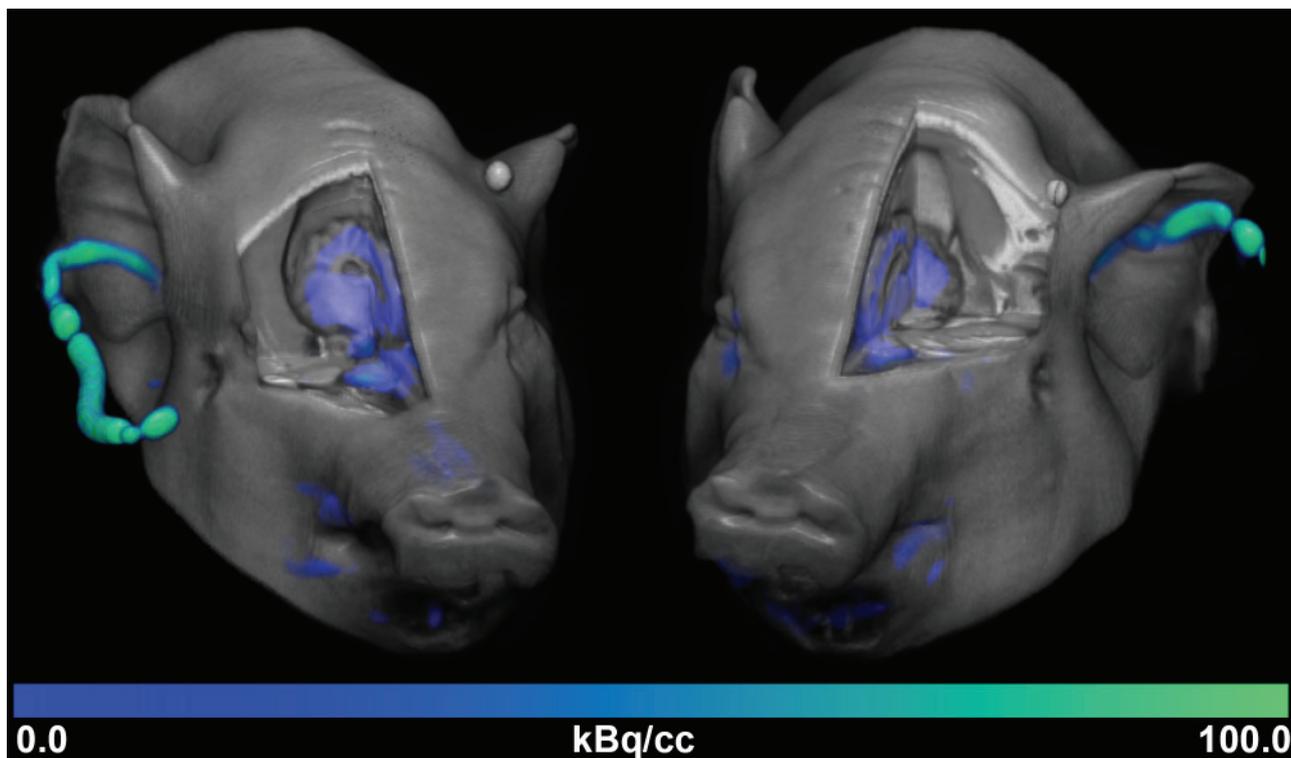


Figure 1. 60 min average PET images co-registered to a MRI image displaying tracer residues in ear vein catheters and connecting tubes from [11C]PK11195 in two Göttingen minipigs after injection of 300-400 MBq tracer in a volume of 10 ml. The catheters were flushed with 10 ml saline after tracer injection.

dence of local random scatter. This might result in so-called streaking or halo artifacts in the images, as is also seen in areas adjacent to structures with high tracer uptake (i.e. bladder or kidneys) (Noto *et al.*, 2017).

Figure 1 shows how tracer residues may appear in [11C]PK11195 PET images. [11C]PK11195 PET is commonly used for detection of neuroinflammation in humans as it binds to the translocator protein 18 kDa (TSPO), which is highly expressed in activated microglia. It has also previously been used in pig imaging studies (Cumming *et al.*, 2001; 2006). Our group recently embarked on studies of neurodegenerative disease in pigs with the [11C]PK11195 PET tracer (data not published). In these studies, although the same amount (300-400 MBq) and volume (10 mL) of tracer was used, the amount of tracer retained in the ear vein differed between individual minipigs. Similar problems of tracer sticking to the internal tubing of catheters have been seen with other tracers. Here we will discuss various methods to avoid or reduce this problem based on our studies with different tracers.

Materials, methods and results

Animals

Our investigations are reported in compliance with the ARRIVE guidelines. All studies were performed on adult female Göttingen minipigs from Ellegaard Minipigs ApS (Dalmose, Denmark) on licences (2016-15-0201-00878 and 2016-15-0201-01138) issued by The Danish Experimental Animal Inspectorate. Minipigs were fed a restricted pellet diet (SDS, Aarhus, Denmark) and fasted overnight prior to studies, with continuous access to tap water. The minipigs were used in different brain research projects (data yet not published), and they were acclimated for several weeks at 20°C and 50-55 % relative humidity prior the studies, with a controlled photo-period of 12 hours light and with air changed eight times every hour. Minipigs were single or group housed in 4.6 m² enclosures with fence-line contact with congeners.

Dilution of tracer prior to injection

Dilution of the tracer prior to injection and flushing with saline after injection are obvious possible options for reducing the residue in the catheter. We have examined the effect of tracer volume and flushing volume in an *in vitro* experiment. We used [18F]

FDOPA, a marker of dopamine synthesis widely used in pig brain studies (Danielsen *et al.*, 2001) which we also have previously observed to adhere to the ear catheters. We injected approximately 100 MBq tracer in 22G catheters (BD Venflon ProSafety 0.9mm x 25 mm; Abena, Aabenraa, Denmark), which initially had been flushed with saline. The tracer was diluted in saline to total volumes of 5 mL, 10 mL, 20 mL and 50 mL. Similarly, the catheters were immediately flushed with 5 mL, 10 mL, 20 mL or 50 mL of saline after tracer injection. Subsequently, the residue of the tracer was measured (one minute/sample) in a gamma counter (Packard Cobra Gamma Counter, Model D5003, GMI Inc, Ramsey, Minnesota, USA), and the residues were calculated as % of the injected tracer dose. The experiment was repeated and gave similar results both times (Figure 2). We found a large effect of diluting the tracer prior to injection (from 0.011-0.015% in 5 mL to 0.002-0.003% in 50 mL volume, Figure 2A), while the flushing volume (5 mL - 50 mL, Figure 2B) had no effect. This suggests that diluting the tracer in a larger volume will result in a smaller portion of the tracer coming into contact with the surface of the catheter and adhering to the plastic surface. However, once the tracer has adhered to the surface it is difficult to flush away with saline, and therefore the flushing volume had no effect in this setup. Consequently, it is unlikely that a saline drip will have a significant impact on reducing catheter-bound tracer residues.

Based on these results, we first increased the tracer volumes from 10 mL to 20 mL or 50 mL in our ongoing minipig studies (data not published). In one study, we found no visible residues in ear catheters when using 50 mL volume of [¹⁸F]fludeoxyglucose

(6 minipigs scanned 3 times = 18 scans), a widely used tracer for the study of glucose metabolism in the brain (Poulsen *et al.*, 1997). The disadvantage of increasing the injected tracer volume to such a large extent is that this increases the injection time and thereby compromises injection of the tracer as a true bolus. Moreover, even though the tracer is diluted in a larger volume, streaking artifacts still occurred during the injection and therefore an investigator should strive for a minimal injection time. The extent to which this is important depends on the purpose of the study, the nature of the tracer and the plans for data quantification and kinetic modeling. Therefore, there is a trade-off between having a fast injection and increasing the injection volume to reduce ear vein residues.

Removing the catheter immediately after injection

Another option is to remove the catheter immediately after the injection of particularly sticky tracers. We have tried this method in an experiment with [¹¹C]PIB, a tracer for detection of beta-amyloid in the brain (Laforce *et al.*, 2011). By removing the catheter immediately after injection, we limited the radiation from the ear during the first minute of the scan. This corresponds to the time it takes to inject the tracer and flush with saline. Furthermore, it has allowed us to measure how much tracer remains in the catheter and not just in the syringe. We have thus found that 0.2% - 0.6% of the injected dose was left in the ear vein catheter. Although the percentage of dose remaining in the catheter is small, it is present in a much smaller volume than the injected dose (i.e. 0.2 mL catheter volume vs 20 L pig volume). There-

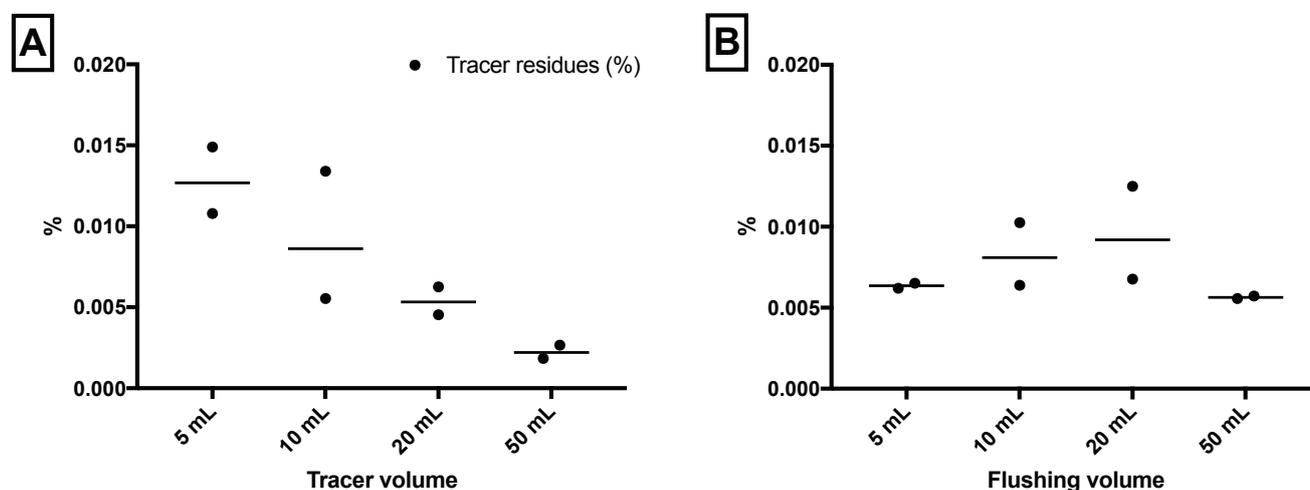


Figure 2. Results of two *in vitro* studies with the tracer [¹⁸F]FDOPA using different tracer volumes (5-50 ml) and flushing volumes (5-50 ml). The residues of the tracer are shown as % of the injected tracer dose.

fore the activity concentration of the tracer remaining in the catheter will be substantial. The disadvantage of removing the ear vein catheter is that it will only be possible to inject one tracer. Therefore, it would limit the possibility to conduct multi-tracer studies. A way to circumvent this issue would be to insert one catheter in each ear vein for multi-tracer studies. Another option, if multi-tracer studies are planned, would be to first inject a tracer that is not sticky (for example, raclopride) prior to injection of the neuroinflammation or protein binding markers that adhere to the catheters. It is important to note that removing the catheter during the first 1-2 minutes of the scan will affect the attenuation scan, and therefore we recommend the attenuation maps to be acquired after the PET scan and the initial minute of the scan to be excluded from the kinetic analysis. Finally, if bleeding is not stopped immediately after ear vein catheter removal, blood-containing tracers can contaminate the skin and so would require attention from a radiation safety viewpoint.

Other injection sites

Instead of using the ear vein for tracer injections, it is possible to catheterize other veins in the pigs. When blood sampling is needed for kinetic analysis, a catheter is normally placed in the femoral artery. As the femoral artery and vein are located close to each other, they can easily be catheterized together with only minor changes to the surgical procedure (Ettrup *et al.*, 2011). A further advantage is that the injection would take place outside the field of view of the PET cameras. The disadvantage is that a femoral vein catheterization requires surgical intervention or ultrasonic guided techniques, which also affects the welfare of the animal. Furthermore, the pig is usually placed on its back in order to gain access to the catheter, which in long-term studies may affect lung function. Our experience is also that scar tissue formation is substantial following a catheterization in the femoral artery, which complicates using the same site twice for injection in longitudinal studies. Other alternatives for accessible veins in the minipig include the peripheral milk vein (Ettrup *et al.*, 2010; Andersen *et al.*, 2015), the cephalic vein or the saphenous veins, but we have not yet used them as injection sites for PET neuroimaging studies.

Image reconstruction

As already mentioned, the artifacts observed in or close to the brain following ear-vein injection were similar to halo or streaking artifacts seen in areas

close the bladder and the kidneys, which tend to accumulate high amount of tracer during a scan. This artificially reduced apparent tracer uptake was probably related to scatter and attenuation correction and might be restored partly by disabling or scaling the scatter correction. However, non-scatter corrected PET images are not quantitative. Reducing the dead time of the detector ring or optimizing the acquisition time could further improve image quality (Lütje *et al.*, 2016), but investigating different image reconstruction possibilities was outside the scope of this paper.

Discussion

Large animal models including pigs are important for translational neuroscience, and PET imaging enables a unique insight into the functions of the healthy and diseased brain. For PET tracer injection, the femoral vein is preferable to the ear vein. Conveniently, the femoral vein can be used in studies where repeated arterial blood sampling is needed, as surgical incision is performed to allow access to the artery. When a non-invasive technique is preferred, the ear vein is a good alternative although it is recommended to dilute the tracer sufficiently prior to injection. As an example, the tracer [18F]FDOPA requires a volume of 20 mL or more to reduce tracer residues, and based on our practical experience with pigs this volume will also help when using other PET tracers. As most tracers used for dynamic PET scanning should be given as bolus injections (< 1 minute), volumes larger than 50 mL are impractical. Moreover, according to the scientific recommendations, a 20 kg pig should as a maximum have a 50 mL intravenous bolus injection (Diehl *et al.*, 2001). The tracer can furthermore be injected through an extension tube (filled with saline before injection), which is removed together with the syringe after tracer injection. This avoids placing the syringe with tracer inside the PET gantry while injecting. The ear of the pig can be secured with tape towards the back along the neck in order to maximum the distance from the brain. Securing the ear has the additional advantage that it cannot move during the injection, which decreases the attenuation correction error in the initial frames of the PET images if the catheter is not removed immediately after injection. By applying these simple techniques it has been, in our experience, possible to obtain high-quality images without exposing pigs to invasive procedures.

Conclusion

We have found that injection of certain PET tracers into the ear vein of pigs can lead to scanning artifacts. In this paper, we suggest measures to reduce these effects. We discuss the benefits of increasing the injection volume, removing the catheter immediately after injection and changing the tracer injection site. Each of these solutions can lead to other study limitations, so a fine balance is required depending on the type of tracer and the purpose of the study (single tracer vs multi-tracer, acute vs. longitudinal, etc).

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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