Dynamic PET Imaging with Arterial Input Function of Lipopolysaccharide Induced Neuroinflammation in Rat

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1 ARTERIAL INPUT FUNCTION (AIF)

Input function is a necessity for dynamic PET imaging and it can be obtained from the PET image (image derived input function) or by arterial plasma sampling. AIF allows multiple compartment models in the kinetic analysis and is a standard procedure in clinical PET centres. Rodent models are widely used in preclinical research, but conventional AIF from rodents with multiple arterial blood samples is impractical due to their limited blood pool. In optimal situation reference tissue models are used with dynamic small animal PET, but for some tracers no reference tissue is available e.g. translocator protein (TSPO) tracers.

Blood radioactivity during PET scan can be measured from continuous flow arteriovenous shunt and flow cell coincidence counter. In the literature, this procedure requires cannulation of femoral artery and vein. The surgery has been a terminal procedure for the imaged rat and longitudinal PET studies with the same subjects have been impossible. These drawbacks have previously limited the use of translational AIF in preclinical imaging.

We have developed a nonsurgical approach for arteriovenous shunt cannulation which can be applied for full AIF generation during PET imaging (Figure 1). The procedure allows longitudinal imaging of the same rat after one week of recovery time.

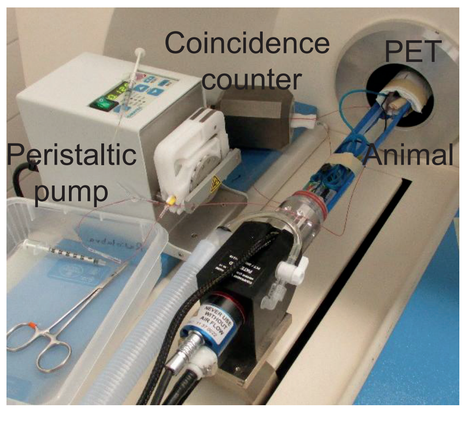

Figure 1. The rat is cannulated in the ventral tail artery and lateral tail vein prior imaging. The blood flow is maintained constant in the shunt with a peristaltic pump during the scan. Radiotracer dosing and blood sampling, for plasma and parent fraction, can be performed trough the shunt. The obtained blood input curve from coincidence counter is further correct with "whole blood – plasma fraction" and "unmetabolized tracer in plasma fraction" to have full AIF for the PET image analysis.

2 IMAGING

Rats were imaged 1, 8 and 15 days post intrastriatal LPS infusion with ^{18}F-FEPPA (30 – 46 MBq) using BioPET/CT small animal scanner (Sedecal, Figure 2). The animals were anesthetized with 5 % isoflurane and maintained under anaesthesia 1.5 – 2 % isoflurane. The tail artery and vein were cannulated and connected to a coincidence counter (Twilite, SwissTrace) and a peristaltic pump with a constant flow rate (0.32 ml/min). Dynamic list mode PET imaging was performed over 90 minutes and blood samples were collected at 3, 6, 9, 12, 15, 20, 30, 45, 60 and 85 min to generate AIF.

The sinograms were reconstructed with 3D OSEM (1 iteration, 50 subsets) applying attenuation correction based on CT image from the animal. Individual AIFs for PET image analysis were generated from each rats own blood input curve corrected with plasma and parent fractions. The analysis was performed with 2-tissue compartment model (2TCM) and Ichise's multilinear reference tissue model (MRTM) with PMOD software (v3.7)

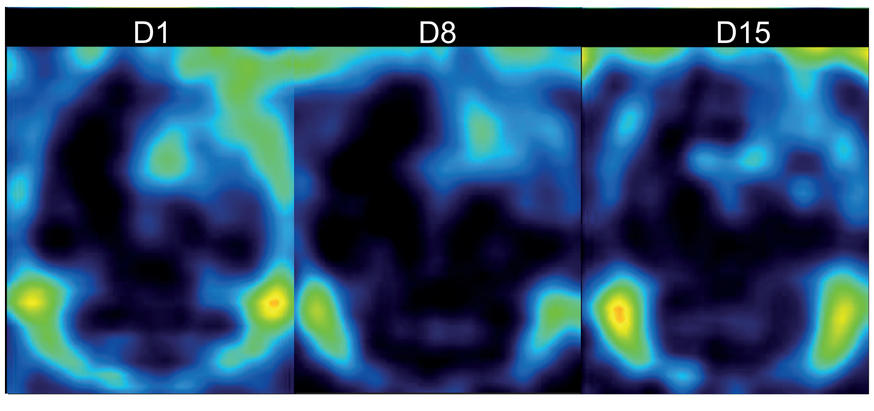

Figure 2. Representative horizontal PET images of unilateral neuroinflammation in rat brain induced with intracranial LPS injection. The rats were imaged 1, 8, and 15 days post LPS infusion. PET images shown as averaged frames 43 – 90 min post ^{18}F-FEPPA injection.

3 GENERATION OF AIF

The whole blood -plasma fraction was measured with gammacounter from whole blood and plasma samples (á 5 µl) collected during the PET scan. Used radiotracer (^{18}F-FEPPA) bound heavily to erythrocytes within the first 5 minutes of the scan and was slowly reversed back to plasma due to conversion to metabolites (Figure 3A).

^{18}F-FEPPA was extracted from plasma samples (á 40 – 50 µl) using acetonitrile (extraction rates 0.85 – 0.97) and the unmetabolized fraction of ^{18}F-FEPPA was separated with radio-TLC (Rf 0.67 – 0.74). Activity analysis was performed with gammacounter (Figure 3B).

Functions of plasma fraction and parent fraction were used to correct individual blood input curves to obtain AIF i.e. unmetabolized concentration of ^{18}F-FEPPA in plasma (Figure 4).

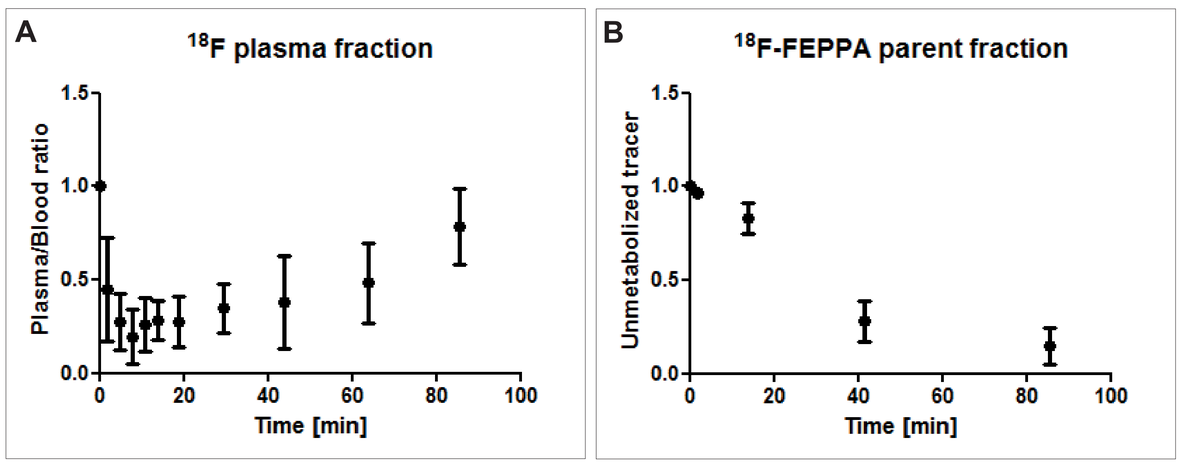

Figure 3. Averaged whole blood – plasma fraction of radioactivity in blood samples during the PET scan (A). Averaged fraction of unmetabolized TSPO tracer (^{18}F-FEPPA) in plasma samples (B).

4 KINETIC PET ANALYSIS

^{18}F-FEPPA accumulation was higher on the LPS infused site up to 15 days after the operation. Injection site is visualized as a bright spot on the right striatum on D1. Later on, the TSPO expression has spread to a larger area on D8, eventually stating to phase out on D15 (Figure 2).

TSPO expression is low in healthy brain. To compare different kinetic models, we applied unilateral LPS neuroinflammation model in rat and hence contralateral side could be used as a reference tissue. Further, results obtained with multicompartment model and reference tissue models were compared. To perform multicompartement model, generation of AIF (Figure 4) is required.

As a result, decrease in binding potential, nondisplaceable binding potential (BP_{ND}) and total volume of distribution (Vt) was observed during the 15 day period (Figure 5). Both models provided similar results. Furthermore, the basal level of TSPO expression was lowest on D1 as seen on the time activity curves (TAC) from contralateral striatum and was slightly elevated on D8 and D15 (Figure 6).

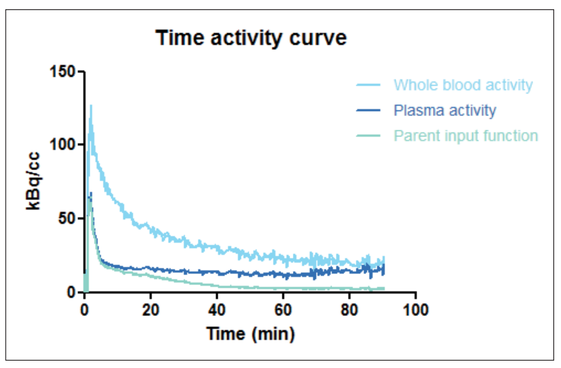

Figure 4. Full AIF is obtained by applying plasma fraction and parent fraction corrections to measured blood input curve. AIF curve is required for multicompartmental kinetic PET analysis.

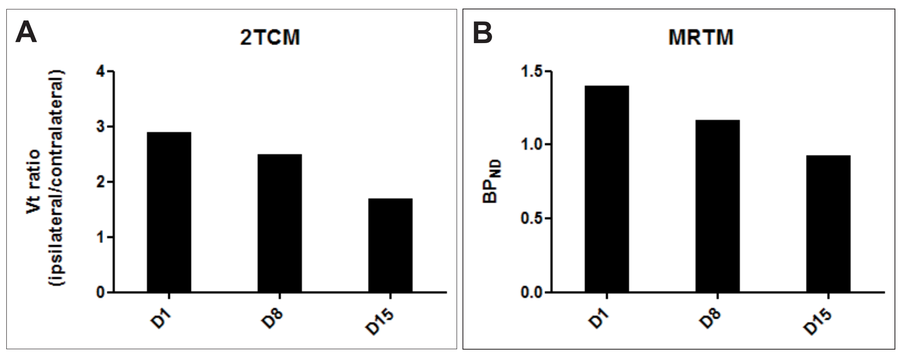

Figure 5. Changes in ipsi- and contralateral striatum Vt-ratio, analysed with two tissue compartments (2TCM, A) and the BP_{ND} of ipsilateral striatum using contralateral striatum as reference tissue, analysed with MRTM (B). Similar change in result was obtained over time with both kinetic models.

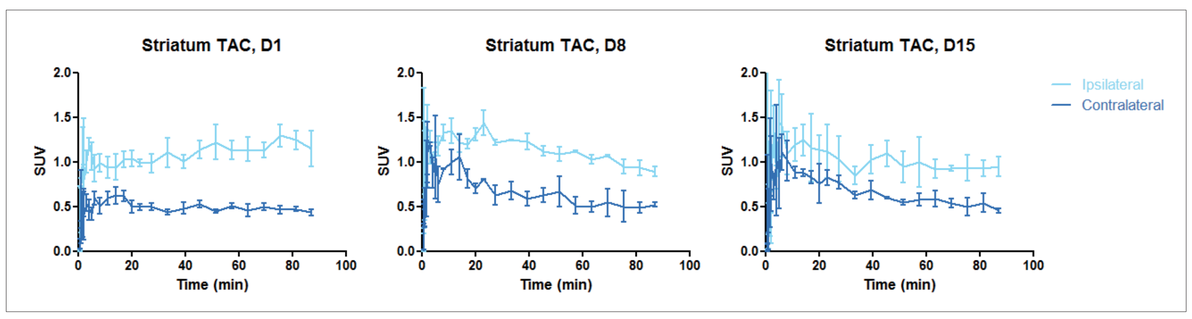

Figure 6. Time-activity curves (TAC) from ipsi- and contralateral striatum 1, 8 or 15 days post unilateral LPS infusion to striatum. The lowest basal level of TSPO tracer (^{18}F-FEPPA) was seen on D1 as the highest binding was seen on D15. The highest activity on infused striatum was seen on D1.

5 SUMMARY

Dynamic PET imaging of neuroinflammation in rodent models is challenging due to several factors. TSPO upregulation is uniform in many neurodegenerative diseases with neuroinflammation. This limits the use of reference tissue models, as no valid reference tissue is available. Traditional techniques for blood sampling to generate full arterial input function (AIF) are not feasible due to limited blood volume of rodents. Significant blood loss may also bias tracer pharmacokinetics. Furthermore, multiple blood sampling has previously conducted only as a terminal procedure preventing longitudinal studies within individual.

With arteriovenous shunt and coincidence counter blood input function can be obtained during dynamic PET scan without significant blood loss. Correction for plasma activity and parent fraction can be generated from minimal blood volume samples collected from the shunt during the scan. This nonsurgical novel approach requires only minimal blood sampling allowing longitudinal PET studies with AIF in rats. i.e. blood loss <10% of total blood volume.

In this study we have imaged longitudinally a rat model of acute neuroinflammation using intracranial lipopolysaccharide (LPS) infusion in the striatum with full AIF. Unilateral LPS neuroinflammation model was selected because contralateral side can be used as reference tissue and comparison to results using multicompartment model and reference tissue models could be compared. Both 2TCM and MRTM models provided similar result on the PET image analysis.

As a summary, applying this novel methodology to generate AIF simultaneously with preclinical PET imaging provides more translational and reliable approach to monitor progression of inflammation applicable for several neurodegenerative rodent models.

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