Translational PET Imaging of Neuroinflammation in Pre-clinical Rodent Models of Neurogenerative Diseases

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1 OVERVIEW

Pre-clinical nuclear imaging provides a translational approach to monitor progression of inflammation can be applied in several rodent models with neuroinflammation. Neuroinflammation is associated with several neurodegenerative diseases, including multiple sclerosis, Parkinson's disease, Alzheimer's disease, Huntington's disease, and stroke. Mitochondrial translocator protein (TSPO) activation and changes in metabolic activity have been associated with neuroinflammation in central nervous system. Therefore TSPO ligands and glucose analogues, radiolabelled with positron emitting isotopes, can be applied to image the progression of neuroniflammation in vivo. TSPO expression in the brain is associated with activation of microglia, therefore TSPO is potential target to evaluate neuroinflammatory changes in a variety of CNS disorders. However, there is always a baseline expression present which prohibits the use of reference tissue models in dynamic PET imaging. To reach optimal readouts from the imaging and understand PET tracer kinetics metabolite corrected arterial input function has to be collected from the imaged animal. Optionally static PET imaging can be applied but static PET imaging data is more prone for variation due to technical aspects and data interpretation has to be made carefully.

Neuroinflammation was studied with TSPO PET and metabolic changes with FDG PET in several animal rodent models of neurodegenerative diseases. In vivo imaging TSPO PET imaging was used to effectively monitor neuroinflammation. Additionally, the metabolic alterations associated with neuroinflammation could be quantified with FDG PET imaging.

As a summary, PET imaging gives multiple options to study neuroinflammation in pre-clinical for CNS disease animal models. It is a powerful research tool allowing comprehensive evaluation of disease progression and treatment interventions in vivo studies.

2 IMAGING

All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by National Animal Experiment Board. Animal facility has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. PET images were reconstructed with 3D OSEM and corrected for attenuation. Image analysis was performed with PMOD (v. 3.7).

3 TRANSLATIONAL QUANTITATIVE PET ANALYSIS

Neuroinflammation after intrastriatal lipopolysaccharide (LPS) infusion, was followed longitudinally in male SD rats 1, 8 and 15 days post operation with translocator protein (TSPO) ligand ^{18}F-FEPPA. To obtain blood input function, 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). During the PET scan blood samples for plasma fraction and parent fraction were collected at 3, 6, 9, 12, 15, 20, 30, 45, 60 and 85 min. The radioactivity was measured in blood and plasma samples (á 10 µl) with gamma counter and unmetabolized tracer in plasma with thin layer chromatography (á 30 – 50 µl). Individual blood input curve was corrected with individual plasma fraction- (Figure 1, A) and parent fraction (B) functions to generate metabolite corrected AIF (C). Total volume of distribution (V_T) values were calculated with 2-tissue compartment model (D).

In the following two weeks after LPS infusion the same individual rat (n=2) was imaged three times including blood sampling for input function. Tracer retention is visible on the injection site suggesting focal neuroinflammation (Figure 2A). Highest V_T values are seen 8 and 15 days post LPS infusion (Figure 1B). The method described here allows longitudinal imaging or same rat with full metabolite corrected arterial input function.

3 TRANSLATIONAL QUANTITATIVE PET ANALYSIS CONT'D

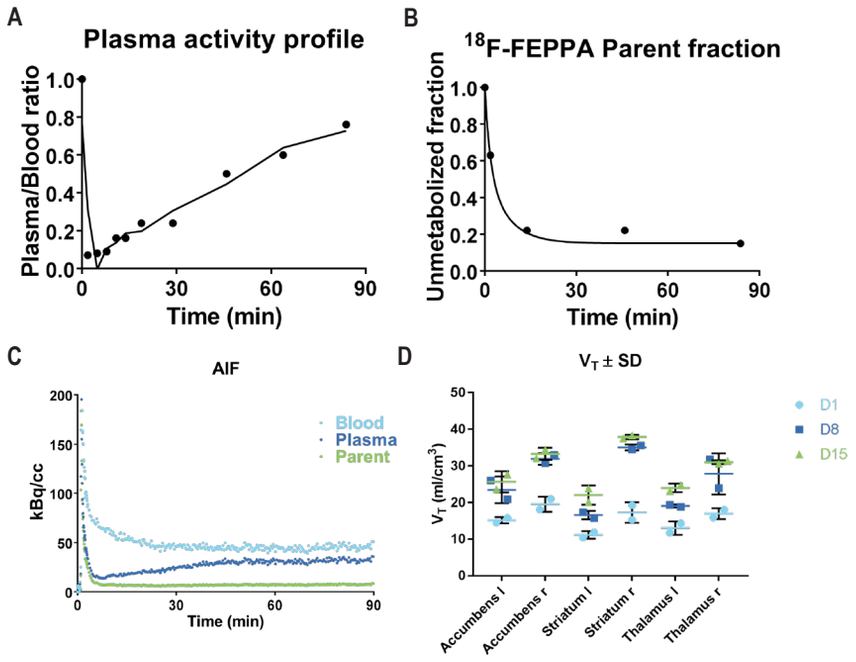

Figure 1. A) Radioactivity distribution between whole blood and plasma during the PET scan in LPS induced rats. B) Fraction of unmetabolized ^{18}F-FEPPA in plasma during the PET scan. C) Generation of AIF from the measured blood input curve corrected with plasma fraction- and parent fraction functions. D) V_T-values of ^{18}F-FEPPA in nucleus accumbensis, striatum and thalamus of both hemispheres. V_T was low on D1, increased thereafter and remained elevated between D8 and D15. Individual data points are plotted in the graphs with mean ±SD.

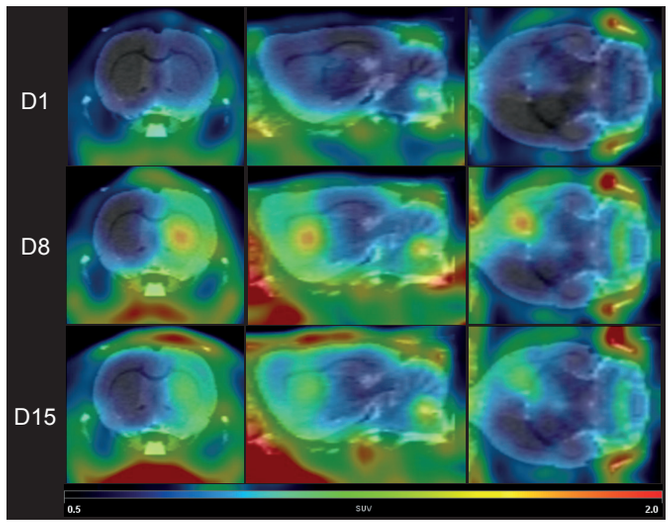

Figure 2. Individual rat imaged 1, 8 and 15 days after intrastiatal LPS infusion with TSPO tracer ^{18}F-FEPPA. Metabolite corrected AIF was generated for each scan. Higher tracer accumulation was seen to the injection site. The PET images (averaged frames 30 – 90 min) were aligned with rat MRI template. Sections shown as coronal, sagittal and horizontal views.

4 NEUROPATHIC PAIN

Neuritis model was used to study perineural inflammation and neuropathic pain both in mice and rats. Induction was done using modified Complete Freund's adjuvant in Oxygel band wrapped around sciatic nerve. Animals did not exhibit mutilation of limbs or demonstrate moribund behavior, or behavior that would otherwise indicate severe spontaneous pain (shaking or licking of paws) during the study.

Mice were imaged with FDG-PET 6 days post surgical operation. Rats were scanned using FDG-PET on days 7 and 21 as well with ^{123}I-CLINDE on day 4 (Figure 3).

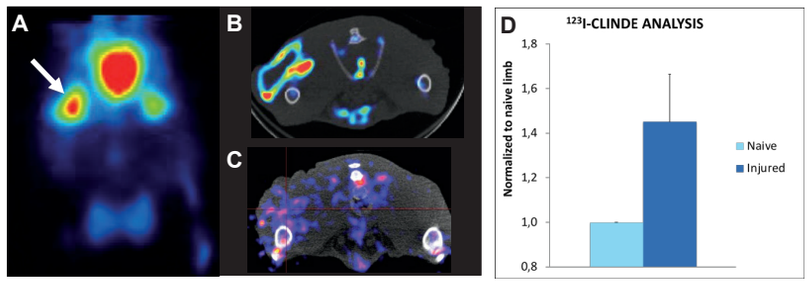

Figure 3. PET and SPECT imaging in neuritis model. In mice 1.5-fold increase in FDG consumption in injured leg compared to naive leg was observed (A). Example FDG-PET (B) and ^{123}I-CLINDE-SPECT (C) coronal images of neuritis model in rat. In rats stable inflammation (ca. 5-fold) was seen 7D and 21D post induction using FDG-PET imaging. In TSPO imaging 1.6-fold accumulation of ^{123}I-CLINDE on day 4 (D) post induction was seen on the operated hind leg.

5 MULTIPLE SCLEROSIS

To study multiple sclerosis C57Bl/6 female mice were given cuprizone (0.3% w/w) in their diet or regular powdered diet. Exposure lasted 6 weeks after which the cuprizone supplementation of the diet was discontinued. PET/CT imaging were performed on week 7. Prior the PET scan, mice were fasted over night. FDG was dosed (i.v., ca. 20 MBq) 35 min prior a static PET scan.

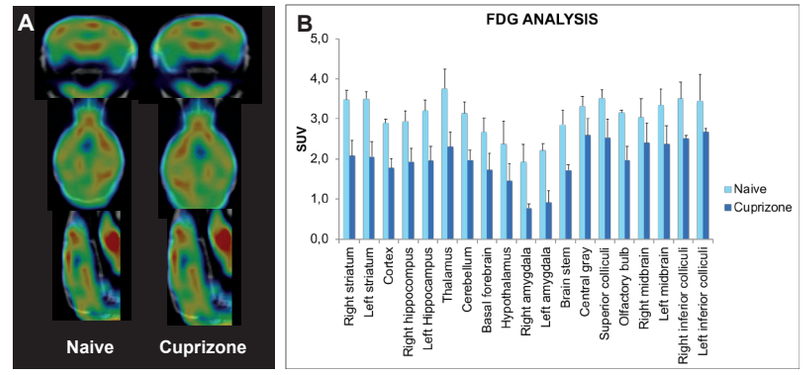

Figure 4. Neuroinflammation was studied in cuprizone mouse model of multiple sclerosis. Example images of in naive and cuprizone induced mouse in coronal, horizontal and sagittal view. Example FDG-PET images of brain metabolic activity in naive and cuprizone mice (A). Significant decrease in brain metabolism was quantified in several brain regions (B). Image analysis results shown as mean +SD. Quantification was done using PMOD.

6 CONCLUSIONS

In this study, we present the utility of translational nuclear imaging applications to study neuroinflammation in pre-clinical rodent models. PETT/CT imaging with ^{18}F-FEPPA has shown to effectively detect neuroinflammation e.g. in LPS related inflammation and neuropathic pain models. Further, metabolic alterations associated with neuroinflammation were also quantified using FDG-PET. As a summary, nuclear imaging of inflammation is a powerful research tool allowing comprehensive evaluation of disease progression and treatment interventions in vivo studies.

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