Nuclear Imaging of Neuroinflammation in Rodent Models of Neurodegenerative Diseases

Tuulia Huhtala[1], Jussi Rytkönen[1], Pekka Poutiainen[2, 3], Laura Tolppanen[1], Anna-Mari Zainana[1], Teija Parkkari[1], Outi Kontkanen[1], Patrick J. Sweeney[1], Antti Nurmi[1]
[1]Charles River Discovery Services, Kuopio, Finland
[2]University of Eastern Finland, Kuopio, Finland
[3]Kuopio University Hospital, Kuopio, Finland

516.14

1 OVERVIEW

Neuroinflammation is associated with neurodegenerative diseases, including multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), and traumatic brain injury (TBI). Activation of the mitochondrial translocator protein (TSPO) in neuronal tissue is linked with neuroinflammation and TSPO ligands can be applied to image the progression of neuroniflammation *in vivo*. Also, changes in metabolic activity have been associated with neuroinflammation.

TSPO is localized in mitochondria of glial cells and expressed in very low concentrations in normal brain. Their expression increases after microglial activation following brain injury. Accordingly, TSPO's are potential targets to evaluate neuroinflammatory changes in a variety of CNS disorders.

^{18}F-FEPPA is a second-generation TSPO ligand with improved penetration, selectivity and suitable metabolic profile. For *in vivo* SPECT/CT imaging of neuroinflammation, ^{123}I-CLINDE has been used as a TSPO radioligand. In the current studies, we utilized these ligands to assess the extent of neuroinflammation in neuropathic pain, induction of MS and lipopolysaccharide (LPS) infusion.

Pre-clinical nuclear imaging provides more translational approach to monitor progression of inflammation and is applicable in several rodent models with neuroinflammation. In addition, dynamic PET imaging combined with real time blood activity quantification from the shunt using a coincidence counter to generate arterial input function (AIF) provides truly translational approach for kinetic modelling where reference tissue is not applicable.

In these studies, *in vivo* imaging using ^{18}F-FEPPA or ^{123}I-CLINDE as TSPO ligand was shown to effectively detect neuroinflammation. In addition, metabolic alterations associated with neuroinflammation were also quantified by using FDG. These results highlight multiple options to study neuroinflammation in animal models.

All animal experiments were approved by the National Animal Experiment Board, Finland. The animal facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International.

2 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 1)

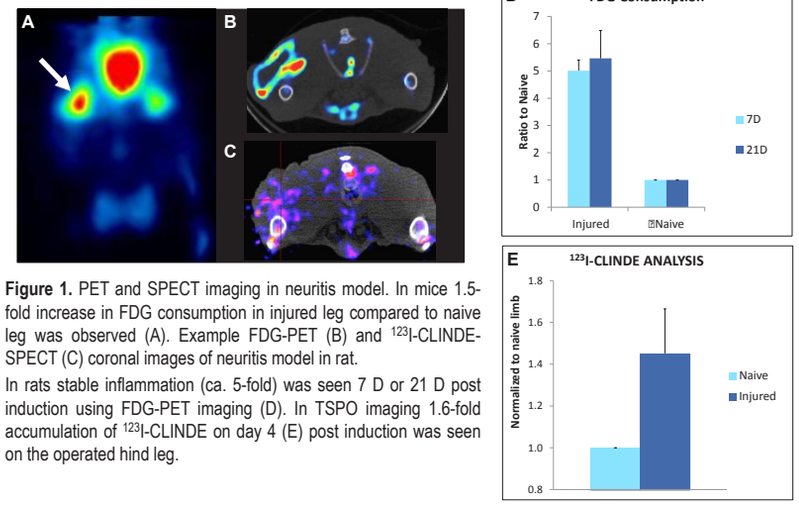

Figure 1. PET and SPECT imaging in neuritis model. In mice 1.5-fold increase in FDG consumption in injured leg compared to naive leg was observed (A). Example FDG-PET (B) and ^{123}I-CLINDE-SPECT (C) coronal images of neuritis model in rat.

In rats stable inflammation (ca. 5-fold) was seen 7 D or 21 D post induction using FDG-PET imaging (D). In TSPO imaging 1.6-fold accumulation of ^{123}I-CLINDE on day 4 (E) post induction was seen on the operated hind leg.

3 MULTIPLE SCLEROSIS

Neuroinflammation was studied in cuprizone mouse model of 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. SPECT/CT and PET/CT imaging were performed on week 7 (Figure 2). After imaging brains were dissected and immediately frozen for autoradiography analysis. Maximal ligand binding (Bmax) to compare density of cannabinoid receptor 1 (CB1) using ^{3}H-Rimonabant was analyzed from globus pallidus and substantia nigra (Figure 3 and Table 1).

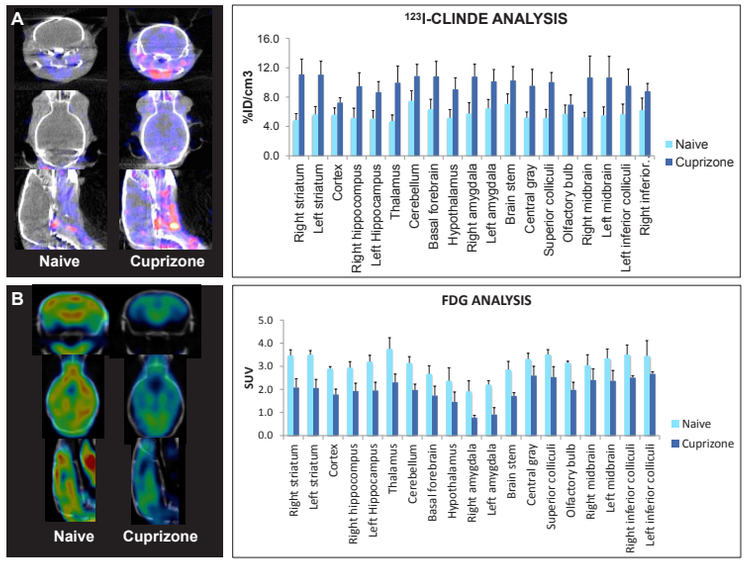

Figure 2. Example images of SPECT (A) in naive and cuprizone induced mouse in coronal, horizontal and sagittal view. Significant ($p<0.05$) increase in accumulation of ^{123}I-CLINDE was quantified in all studied brain regions. Example FDG-PET images of brain metabolic activity in naive and cuprizone mice (B). Significant decrease in brain metabolism was quantified in several brain regions. Image analysis results shown as mean +SD. Quantification was done using PMOD.

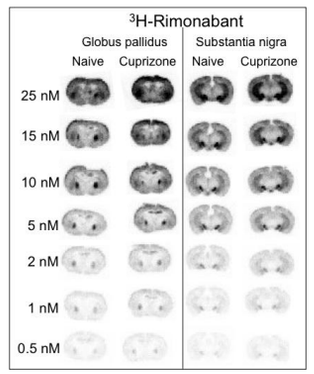

Figure 3. The purpose of this experiment was to compare density of cannabinoid receptor 1 (CB1) in naïve cuprizone mouse model. To compare receptor density, Bmax of ^{3}H-Rimonabant was analyzed from globus pallidus and substantia nigra using real-time scintillation counter (BetaImager). Scanning time of samples was 6 – 16 h. For more information on autoradiography, see posters 751.07 and 226.01.

Table 1. Significant ($P<0.05$) decrease in Bmax of cuprizone mice compared to naive in globus pallidus was seen but no difference in CB1 binding to substantia nigra was seen.

Brain Area	Change in CB1, cuprizone vs. naive	Significance (F-test)
Globus pallidus	-27.8 %	0.041
Substantia nigra	-6.7 %	0.676

4 RELEVANCE OF ARTERIAL INPUT FUNCTION IN PET ANALYSIS

Input function is a necessity for dynamic PET analysis and it can be obtained from 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 with multiple arterial blood samples is impractical due to limited blood volume. In optimal situation reference tissue models are used with dynamic small animal PET analysis, but e.g. for translocator protein (TSPO) ligands reference tissue is not available.

Blood radioactivity during PET scan can be measured as continuous flow from surgically operated arteriovenous shunt connected with flow cell coincidence counter Twilite (SwissTrace, Switzerland). We have applied cannulation to tail artery and vein which can be applied for full AIF generation during PET imaging.

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 image analysis. In this study imaging was longitudinally performed in acute neuroinflammation inflected with an intracranial lipopolysaccharide (LPS) infusion in the rat striatum with full AIF. The radiotracer used was second generation TSPO ligand ^{18}F-FEPPA.

For more detailed information about AIF imaging, visit poster 516.11.

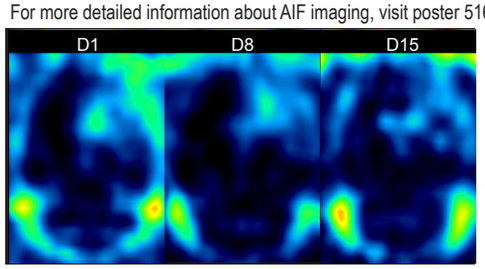

Figure 4. Representative horizontal PET images of unilateral neuroinflammation in rat brain induced with intracranial LPS injection. The rats were imaged 1, 8, and 15 days post LPS infusion. Pet images shown as averaged frames 43 – 90 min post ^{18}F-FEPPA injection.

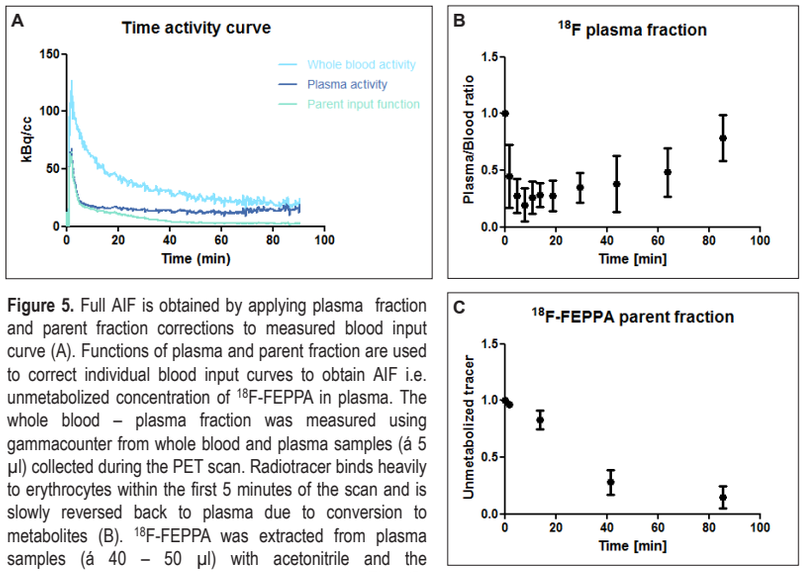

Figure 5. Full AIF is obtained by applying plasma fraction and parent fraction corrections to measured blood input curve (A). Functions of plasma and parent fraction are used to correct individual blood input curves to obtain AIF i.e. unmetabolized concentration of ^{18}F-FEPPA in plasma. The whole blood – plasma fraction was measured using gammacounter from whole blood and plasma samples (á 5 µl) collected during the PET scan. Radiotracer binds heavily to erythrocytes within the first 5 minutes of the scan and is slowly reversed back to plasma due to conversion to metabolites (B). ^{18}F-FEPPA was extracted from plasma samples (á 40 – 50 µl) with acetonitrile and the unmetabolized fraction was separated with radio-TLC (Rf 0.67 – 0.74) and measured with gammacounter (C).