Cuprizone Model in Mice: Characterization of White Matter, Tissue Pathology and Inflammation by MRI, PET and SPECT

KIMMO LEHTIMÄKI, TUULIA HUHTALA, ANNA-MARI ZAINANA, ROBERT HODGSON, ANTTI NURMI

Charles River Discovery Services, Kuopio, Finland

PRESENTATION NUMBER 223.03

BACKGROUND

Multiple sclerosis (MS) and other demyelinating diseases are signficant burden to society and cause life long discomfort and suffering. Although there has been advances in drug development to combat these devastating human diseases, complex nature of the diseases and hetrerogenous patient population demands better and safer therapies. Animal models like experimental autoimmune encephalomyelitis (EAE) and curprizone induced demyelination model are commonly used for preclinical efficacy assessments in early drug development. We have previously demonstrated the benefit of small animal imaging in multiple animals models of CNS disorders, including EAE. Using the same approach we describe here three different imaging applications applied in cuprizone model for demyeliation in mice to supplement more conventional research methods such as behavioral assessments, histopathology and biochemistry. Findings described here are part of the broader characterization and validation efforts of mouse cuprizone model widely used for MS studies and other demyelinating disorders.

MATERIALS AND METHODS

Animals. All experiments were performed under approval by the National Animal Experiment Board, Finland. Female C57Bl/6J mice were used for the experiments, staring at the age of 8-10 weeks of age upon cuprizone challenge.

Cuprizone challenge and maintenance. Mice were challenged with cuprizone ([Bis(cyclohexanone)oxaldihydrazone] 0.3% (w/w) mixed in dry powdered regular diet and provided on the home cage floor. Diet supplemented with cuprizone was added according consumption daily and consumption was monitored as accurately as possible during the 6 week challenge period. Naive female mice received same regular diet in same powdered form as cuprizone mice throughnout the duration of experiments. These experiments were part of the overal cuprizone model characterization effort combining behavioral, imaging, immunohistochemical, immunophentoyping and biomarker end-points.

SPECT/CT. [^{123}I]-TSPO tracer (Clinde, MAP Medical, Finland) was used for neurinflammatory profiling in mice subjected to cuprizone challenge for 6 weeks. Animals are anesthetized with isoflurane using 70:30 N_2/O_2 as carrier gas. SPECT imaging was started ca. 30 min post injection of [^{123}I]-TSPO tracer (ca. 15 MBq/mouse) using NanoSPECT/CT. Imaging protocol consisted of planar tomography image (55 kVp, 500 ms exposure time) which was used as a reference to choose imaging area. After choosing the imaging area, helical SPECT imaging was performed from the same coordinates using 150 s / time frame (head). High resolution multipinhole apertures (NSP-101-M12-WB for mouse) were used to enhance resolution. After SPECT imaging helical CT was performed (180 projections, 55 kVp, 550 ms exposure time). HiSPECT reconstruction was used for the SPECT images. Image processing was performed using InVivoScope or PMOD. For the analysis activity concentration (%ID/cm3) was counted for brain structures according to MRI co-registered template (PMOD).

PET/CT. The animals are fasted overnight prior to ^{18}F-FDG (MAP Medical, Finland) administration and PET imaging to stabilize glucose metabolism. Animals were kept on a heating mat (+37 °C) over night and prior scanning. For PET imaging animals injected with ca. 20 MBq of ^{18}F-FDG i.v. via tail vein under isoflurane anesthesia. Animals were kept awake prior to scanning, anesthesia was re-started 45 min post FDG dosing. Static PET scan (20 min, 250 – 700 keV) was started immediately after placing the animal into scanner. PET images were reconstructed with 3D OSEM and FDG uptake was analyzed according to MRI co-registered template (PMOD).

DTI-MRI. Satellite group of animals for controls and cuprizone challenged animals at 3 and 6 weeks post-treatment start were sampled for ex vivo DTI measurements. PFA perfusion fixed brains in skull were embedded to proton-free imaging medium (GALDEN, perfluorinated liquid, Solvay Solexis, Italy) and imaged using 11.7T small animal MR system (Bruker BioSpec, Ettlingen, Germany). Following parameters were used for 2D 4-segment SE-EPI DTI sequence: Field-of-View 20x10 mm^2, matrix 256x128, twenty-four 0.5 mm thick slices, TE = 25.4 ms, TR = 6 s and 4 transitions. Thirty diffusion directions were acquired with *b*-values of 0 and 670 s/mm^2 for control and diffusion weighted images, respectively. Data were converted from Bruker vendor format to NIfTI-1 format and tensor parameters were determined using FSL diffusion toolbox. Fractional anisotropy values were extracted from manully determined ROI's in major white matter structures using MATLAB.

Statisical analysis. All values are presented as mean ± standard deviation (SD) or standard error of mean (SEM), and differences are considered to be statistically significant at the P<0.05 level. Statistical analysis is performed using StatsDirect statistical software. Statistical comparisons were made between cuprizone treated and control (naive) mice by using t-test or Mann-Whitney U-test, when applicable.

RESULTS

Study outline and Imaging end-points

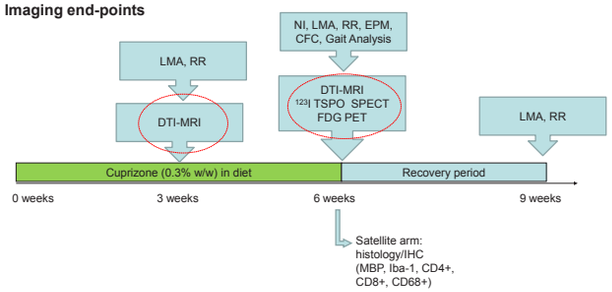

Figure 1. Study schedule for various actoivties in cuprizone model caharaterizartion. Imaging activities were performed at 3 (DTI-MRI) and 6 weeks after the onset of cuprizone challenge.

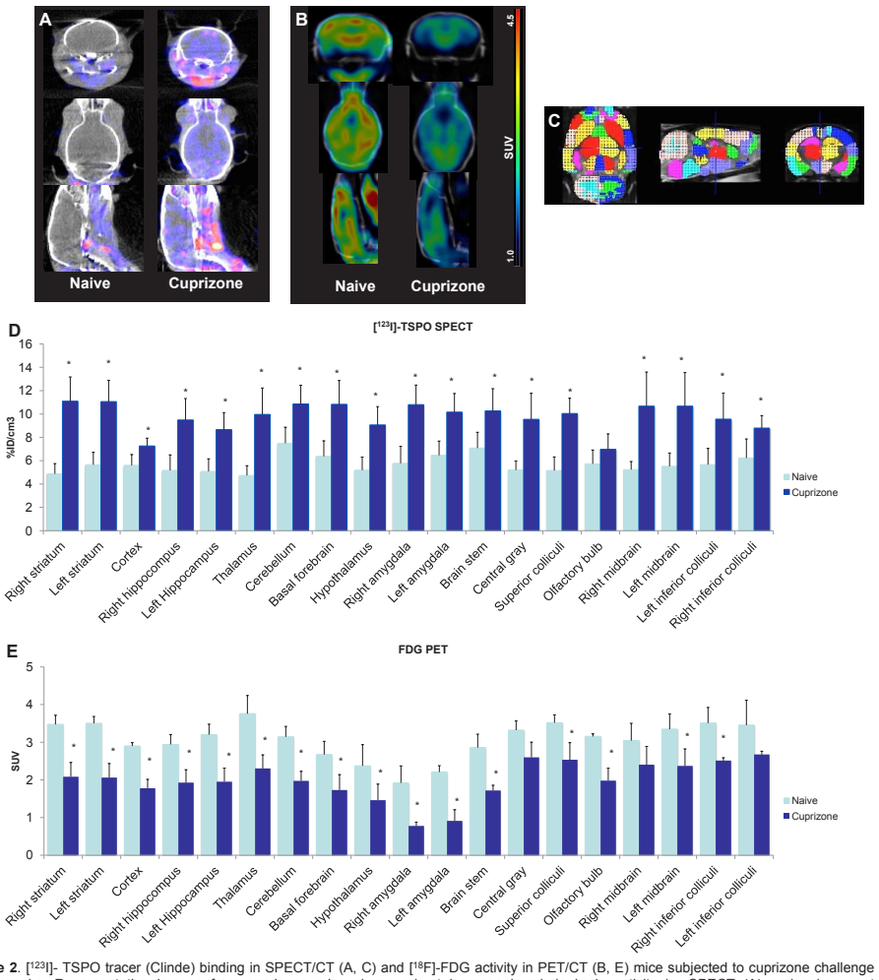

Figure 2. [^{123}I]- TSPO tracer (Clinde) binding in SPECT/CT (A, C) and [^{18}F]-FDG activity in PET/CT (B, E) mice subjected to cuprizone challenge for 6 weeks. Representative images from cuprizone mice shows robust increase in whole brain activity by SPECT (A) and subsequent quantification (D) of the signal in various brain regions using a brain template (panel C, PMOD). In contrast, cuprizone challenged mice showed robust decrease in metabolic activity by PET from various brain regions which was quantified (D) using similar brain template as for SPECT. Dat are presented as mean ± SEM. *p<0.05, t-test between naive vs. cuprizone challenged mice

RESULTS

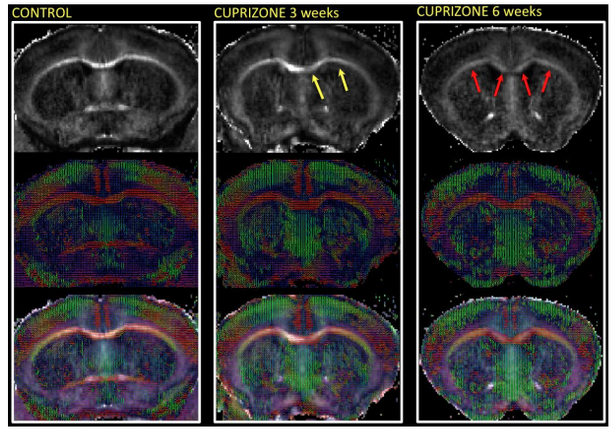

Figure 3. Diffusion tensor magnetic resonance imaging (DTI) from control, 3 weeks and 6 weeks cuprizone challenged animals. Control images show normal homogenous distribution of high FA values in a corpus callosum, whereas cuprizone challenge for 3 weeks show unilateral and partial loss of FA coherence (yellow arrows) and 6 weeks cuprizone treatment leads to a situation, where FA is reduced in whole corpus callosum and this reduction extends to external capsule (red arrows). Colorcoded diffusion principal directions are shown in second row and on bottom row principal directions are overlaid on FA maps.

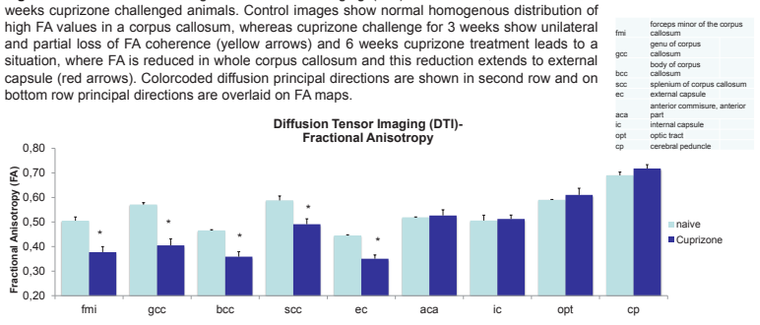

Figure 4. Diffusion tensor magnetic resonance imaging in cuprizone and control mice at 3 and 6 weeks. Quantification of white matter fractional anisotropy (FA) showed signficant decrease in FA values accross multiple white matter rich structures

CONCLUSIONS

This presentation describes our findings from small animal imaging approaches during the cuprizone challenge. The model shows robust behavioral deficits during the challenge period and also histopathological characteristics which are in line with theimaging findings here.

Key findings from these experiments are:

- Cuprizone challenged mice show signficant and relatively global increase in [^{123}I]-TSPO tracer binding in the brain. Substructural analysis of the activities analysed showed sinficant increase in TSPO ligand in essentially all structures analyzed by using co-registered brain template
- Cuprizone challenged mice showed signficantly reduced FDG consumption, accross multiple brain substructures suggesting sugnificantly reduced metabolic activity as a result of cuprizone challenge.
- DTI-MRI showed significant reduction in fractional anisotropy in majority of the white matter rich structures evaluated suggesting robust demyelination during the cuprizone challenge, both at 3 (data not shown) and 6 weeks.

Taken together, our findings show clear and robust changes in inflammatory tracer (TSPO) signal, metabolic activity and white matter content in cuprizone model for multiple sclerosis/demyelination. These findings support and supplement conventional reserach methods, such as histopathological and biochemical analysis methods typically used in the model. These results also showcase preclinical imaging as a powerful non-invasive tool to explore pathological events in the animal models of neurodegeneration allowing more in depth understading of the animal models.