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In Vivo Imaging of a Cy5.5 Labeled Amyloid-β, an Alzheimer's Disease Associated Protein

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Introduction

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder which causes dementia, or loss of global cognitive function. It is reported that over 10% of people in the US over 65 years of age have AD[1]. The intense scientific interest that AD has generated in recent years reflects the prevalence of this disease.

AD pathogenesis is widely believed to be driven by the accumulation of amyloid-β peptide (Aβ), due to the overproduction of Aβ and/or the failure of clearance mechanisms[2]. Aβ is a peptide of 36–43 amino acids which aggregates into small clusters, travels freely in the brain, and deposits into plaques which are hallmark lesions within the brain parenchyma of Alzheimer patients.

Aβ brain uptake and clearance have been studied using radioisotope-labeled Aβ peptides injected directly into the brain, necessitating the use of radioactive tracers for imaging. More recently, fluorescence-labeled Aβ peptides have been used to provide an efficient and simple method for imaging the dynamics of Aβ trafficking *in vivo* within a mouse.

Here, the iBox® Explorer™2 Imaging Microscope (UVP, LLC) was used to visualize the Aβ trafficking *in vivo* with injected Cy5.5-labeled Aβ. The iBox Explorer[2] offers the flexibility to view an entire small animal down to individual cells, both subcutaneously and within the body cavity. The upright optics provide an ultra-long working distance and high numerical aperture for detailed fluorescent-based *in vivo* imaging.

Figure 1. iBox Explorer Imaging Microscope

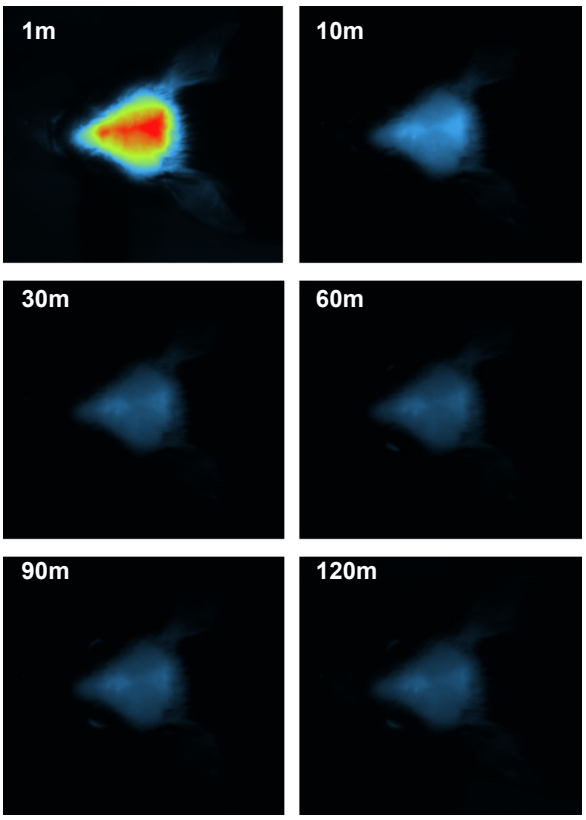

Figure 1. Time course of Cy5.5 accumulation and clearance within the brain of a wild type mouse. From top left to lower right: 1, 10, 30, 60, 90, 120 minutes.

Materials & Methods

Optical Imaging

Nude mice were injected via the tail vein with free Cy5.5 dye (~78μg in 200μL volume) or Cy5.5-labeled Aβ1-40 (100μg in 200μL volume) and were imaged at different time points post injection. To ensure that the imaged fluorescent signal was from Cy5.5-labeled Aβ peptide instead of from the fluorescent tracer itself, both free Cy5.5 dye and Cy5.5-labeled Aβ1-40 were injected into mice and imaged.

The animals were anesthetized with inhaled isoflurane (4% for induction and 1.5% for maintenance) and the hair was shaved from the head and dorsal side of the body. The labeled peptides (100µg) or Cy5.5 free dye (~78µg) were injected intravenously via the tail vein. The animals were then imaged at 0, 1, 10, 30, 60, 90 and 180 minutes post-injection.

Image capture was performed using the iBox Explorer[2] Imaging Microscope. Mice were placed on the imaging stage, maintained at 37°C, for the duration of each imaging experiment. Image acquisition was accomplished using the OptiChemi 610 CCD camera, cooled to 55°C below ambient, and an optical system consisting of interchangeable custom lenses. Bright field and fluorescence images were captured separately at 1x1 binning for each time point with a neutral density and a 710BP40nm emission filter, respectively. Excitation of Cy5.5 utilized the BioLite™ Xe MultiSpectral Light Source (UVP, LLC) with a 630/45 nm band pass excitation filter.

Image Processing and Analysis

Each NIR fluorescence image was histogram-adjusted and measured for optical density using VisionWorks®LS Acquisition and Analysis Software (UVP, LLC). To quantitate the signal intensity for each image, an area density measurement window was selected for a region of interest (ROI) on each raw image. Background intensity was then subtracted from this value to calculate mean density.

Results and Discussion

Time course data shows rapid uptake and clearance of the Cy5.5-Aβ conjugate within the brain of a wild-type mouse. The mouse highlighted in **Figure 2** had an intact blood brain barrier (BBB), a cellular barrier between the vasculature and the brain that is restrictive to most macromolecules. Data using free Cy5.5 did not reveal uptake in the brain (data not shown). However, when the dye was conjugated to Aβ, the dye was rapidly taken up by mechanisms which actively transport Aβ from the bloodstream to the brain and achieved a nearly 8-fold increase. In addition, mechanisms were present which cleared the dye-conjugate from the brain. Semi-quantitative analysis (**Figure 3**) confirms the rapid uptake and clearance of the conjugate, and achieves a nearly 8-fold decrease in the presence of the dye in the brain.

These data confirm the utility of fluorescent imaging technology in elucidating mechanisms involved in the uptake and deposition of Aβ into the brains of study animals. Studies such as these, using fluorescence technology, can form the foundation for future AD studies and thereby lead to a better understanding of the pathophysiology of Alzheimer's disease.

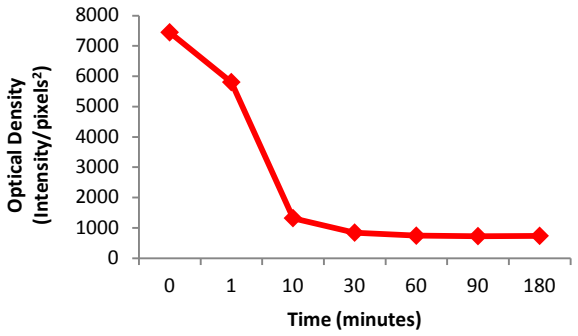

Figure 3. Semi-quantitative analysis of Cy5.5-Aβ conjugate within the brain of a wild-type mouse.

[1] Alzheimer's Association. 2013 Alzheimer's disease facts and figures, PDF document accessed November 1st, 2013.

[2] Molecular mechanisms of neurodegeneration in Alzheimer's disease. Leslie Crews and Eliezer Masliah. Hum Mol Genet. 2010 April 15; 19(R1): R12–R20.