Targeting Endogenous Tau in Human Seeded Tauopathy Models of Neurodegeneration with a Non-Human Antibody

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Abstract

Currently there are 10 immunotherapies targeting tau as a therapeutic target in Tauopathies such as Alzheimer's Disease in Pick's disease under development (Ji, Sigurdsson, 2021) However, the mechanism by which tau antibodies decrease pathological aggregates of tau is unclear. In this study we evaluate antibody-based immunotherapy-like treatment using mouse tau specific antibody mTau8. Our findings demonstrate the antibody's highly selective properties to mouse tau, its capabilities in entering the cerebrospinal fluid by bypassing the blood-brain-barrier, and its ability to decrease tau pathology on the scale of 83.2-91.2% at a 4.5-month timepoint in mouse models. Analysis of these findings, with the mouse brain connectome in mind, reveals a "global" reduction of tau reduction and suggests a mechanism of reduction independent of the cell to cell spreading of pathological tau species. Experimental results in vivo will confirm mTau8's capabilities in reducing tau pathology and live imaging of the cells treated with fluorescently labeled human ADphfs and labeled mTau8 shows statistically significant colocalization.

Background

Hyperphosphorylated aggregations of microtubule associated protein tau is associated with several neurodegenerative diseases collectively classified under the term tauopathy. Markers of neurodegeneration such as phosphorylated tau species (p-tau217 and p-tau181) and co-pathologies such as Amyloid Beta plaques (in familial AD) can be detected as early as 2 decades before the appearance of aggregated tau pathology and the onset of clinical symptoms. Progression of neurodegeneration corresponds more closely with the appearance of aggregated tau as neurofibrillary tangles (Barthélemy et al.,2020). This suggesting a critical role of neurofibrillary tangles and emphasizing its potential as therapeutic targets for the treatment of AD post onset.

In pathological conditions, soluble tau forms misfolded cores that function as the nucleus of tau fibrils. The nucleus recruits physiological tau resulting in the growth and extension of large filaments, depleting the physiological and converting them into the pathological form (Huseby et al., 2019). Pathological tau can also propagate between cells along neural networks. Evidence suggests that cells can release pathological tau and can be internalized by interconnected neurons thus propagating the pathological tau to the interconnected cell (Gibbons et al., 2019).

Contemporary models of tauopathies utilize stereotaxic injections of pathogenic human tau seeds from post-mortem patients into the hippocampus of mice. The pathological human tau would then "seed" the endogenous mouse tau and initiate disease progression similar to sporadic tauopathies independent of mutant tau overexpression in vitro and in vivo (Jing et al. 2016).

While prior studies have analyzed immunotherapy-like-approaches in transgenic mice expressing human tau, the use of human antibodies in transgenic mice fails to discriminate the injected tau seeds and the endogenously expressed Tau (gibbons 2020). To gain insights into the mechanism of tau immunotherapies, we will use a mouse tau antibody (mTau8) to specifically target the endogenous mouse tau without affecting the activity human tau seeds in vivo and in. Evaluating the effectiveness of this treatment in the mouse brain and in mouse primary neuron models will elucidate the mechanism by which an immunotherapy-like-antibody treatment affects disease progression at a post-diagnosis timepoint.

Direct ELIZA

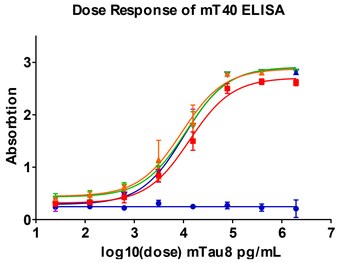

Figure 1: recombinant mouse tau (mT40) is used to detect concentrations of mTau8 at varying doses. These dose response curves demonstrate the sensitivity of the ELIZA to pg of mTau8 per mL with a number of different mT40 concentrations used to plate them.

Results

Antibody Characteristics

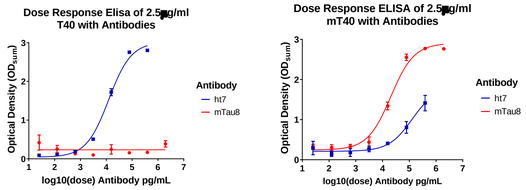

Figure 2-3 : Antibody mtau8 demonstrates an extremely high specificity towards Mouse Tau and has little to no affinity towards human tau. A relatively well known human tau antibody Ht7 is included as a reference demonstrating mTau8's species affinity

CSF and Plasma

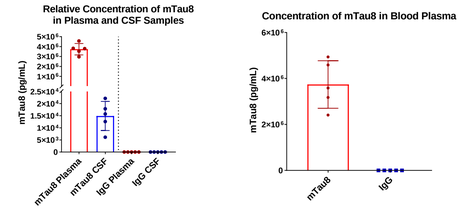

Figure 3-4: Use of the mTau8 direct ELIZA on mouse plasma sample demonstrates that no tau antibodies exists in the blood of IgG-2a control samples. Comparison with CSF samples demonstrate the mTau8 antibody's permeability to the blood brain barrier

Histochemistry

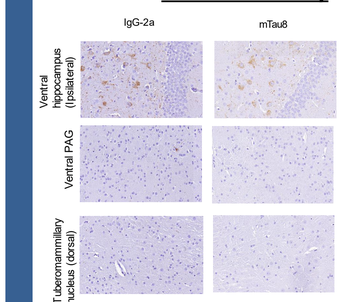

Figure 5: Mice treated with mTau8 demonstrate a reduced tau burden in high connectivity areas such as the Ventral Hippocampus. Analysis via computational methods are used to determine the reduction in phosphorylated tau

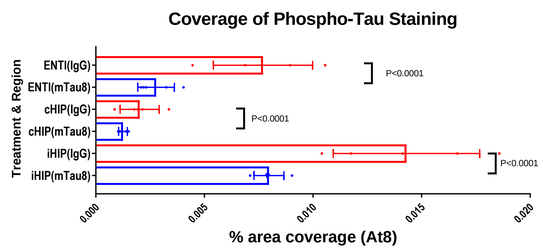

Figure 6 : Mice treated with mTau8 antibody demonstrate significantly reduced phosphorylated tau staining in areas associated with tauopathy spread, namely the Entorhinal cortex, Ipsilateral, and Contralateral Hippocampus.

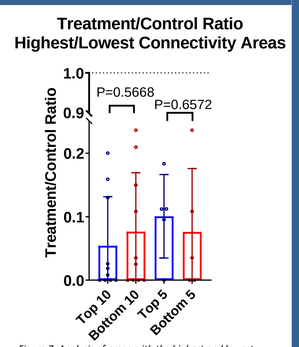

Figure 7: Analysis of areas with the highest and lowest connectivity demonstrate no significant difference in Treatment: Control ratio. Potentially indicating a "global" nature to the reduction of tau.

Cells

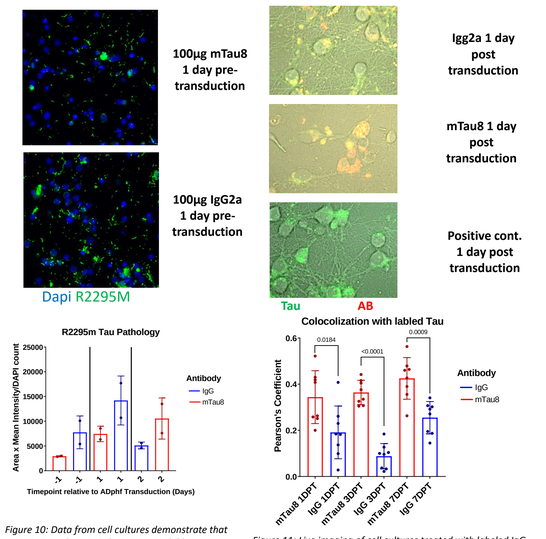

Figure 10: Data from cell cultures demonstrate that treatment with mTau8 decreases Insoluble Tau pathology compared to a control treated with IgG

Figure 11: Live imaging of cell cultures treated with labeled IgG, mTau8, and Igg2a demonstrate colocalization of mTau8 with Tau in live cells at different timepoints

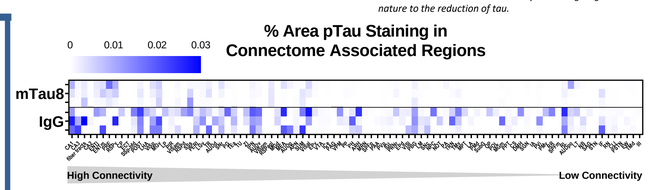

Figure 8: A heatmap of high and low connectivity areas enables a visual representation of the reduction in Tau pathology relative to the connectome.

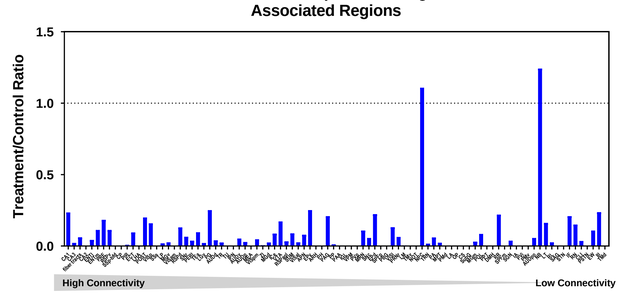

Figure 9: Treatment: Control ratio enables a quantification of the reduction in tau pathology across all areas of the mouse brain according to the regions broken down in the Allen mouse brain Atlas. No significant trend is observed between High and Low connectivity region. On average, the T/C ratio of all regions is 0.1277±0.03962. Translating to a roughly 83.2-91.2% decrease in pathology.

Discussion

mTau8 antibody is a monoclonal mouse anti-rodent tau antibody developed and provided by Janssen Pharmaceuticals. To characterize mTAU8, we used a direct ELISA and selected HT7, a monoclonal mouse anti-human tau antibody, as a control due to its high affinity towards human tau and its relative familiarity in the field. This ELISA is coated with either mT40 recombinant mouse tau or T40 recombinant human tau to detect varying dilutions of mTau8 or HT7. Serial dilutions of each antibody were used to create a roughly linear range of detection. Our results demonstrated that mTau8 had a high affinity towards mT40 and no affinity towards T40 while HT7 exhibited a high affinity towards T40 and mild affinity towards mT40. (Figure 2-3)

In order to assess its effectiveness as a treatment, 22 mice were injected with 10ug of ADphfs derived from human post-mortem tissues. Intraperitoneal injections of mTau8 or Igg-2a were performed one-week post-injection every week for 16 weeks. Using histochemistry, major areas in the contralateral Hippocampus, ipsilateral hippocampus, and ipsilateral entorhinal cortex, Mice treated with Igg were found to have a higher pathogenic load (figure 6). When analysis was increased to account for the entire mouse brain, nearly all regions were found to have a decreased pathogenic load in mTau8 treated mice (figure 8,9). The majority of regions have Treatment vs Control ratios (T/C ratios) below 1 and no clear trend of increasing or decreasing T/C ratio is apparent. When comparing the 5 regions most and least implicated in the connectome, and the 5 least implicated, average T/C ratios demonstrate no significant difference. Extending this to the 10 regions per side maintains the same trend as reflected by p values (figure 7). These results demonstrate a connectome-independent decrease across all regions and suggest a mechanism of action that produces a "global" reduction of phosphorylated tau pathology. On average, the T/C ratio of all regions is 0.1277±0.03962. Translating to a roughly 83.2-91.2% decrease in pathology.

In order to assess the characteristics of mTau8 in bypassing the blood brain barrier, the previously discussed direct ELISA with mT40 was used to measure antibody concentrations of cerebrospinal fluid (CSF) and plasma samples These samples were run in the direct ELISA alongside a known dilution series of mTAU8 in protein buffered saline. In the blood plasma, 3738918 +- 3024446 4453390 pg/ml of mTau8 was detected and 0pg/ml of antibody with affinity to mt40 was detected in the Igg control. In the CSF samples, 14891 +- 7440 22341 pg/ml of mTau8 was detected and 0pg/ml of antibody with affinity to mt40 was detected in the Igg control. These concentrations of mTau8 in the CSF and Plasma correspond to a 0.4% dose of the antibody entering the CSF and demonstrate favorable antibody characteristics.

In vitro mouse neuronal cell culture models were used to further analyze and confirm results in vivo. Mouse neuronal cell cultures were stained with r2295m antibody for insoluble tau pathology and DAPI for cell nucleus staining. Cell cultures were treated with 1000ng/µl in 100µl volume of mTau8 or IgG2a control at 3 different time points, specifically one day before, one day after, and two days after the transduction of 50ng of ADphf was transduced. Preliminary results demonstrate a significant decrease in tau pathology across all time points relative to the IgG2a control (figure 10). In addition to these results, cells were treated with red labeled mTau8 and IgG2a one day prior to the transduction of green labeled ADphf. Live imaging was performed at 1, 3, and 7 days post transduction of the ADphf. Analysis of the results demonstrate statistically significant differences in Pearson's coefficients indicating mTau8's higher colocalization with the labeled Human AD Tau samples (figure 11). Combined with the prior results, a possible explanation could indicate antibody treatments for endogenous tau could result in the clearance of pathogenic Tau and reveal a potential target for future and existing immunotherapy treatments.

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