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Use of LC-SRM method to characterize a mouse model and evaluate an aggregation TAU inhibitor treatment effect on mouse brain

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INTRODUCTION

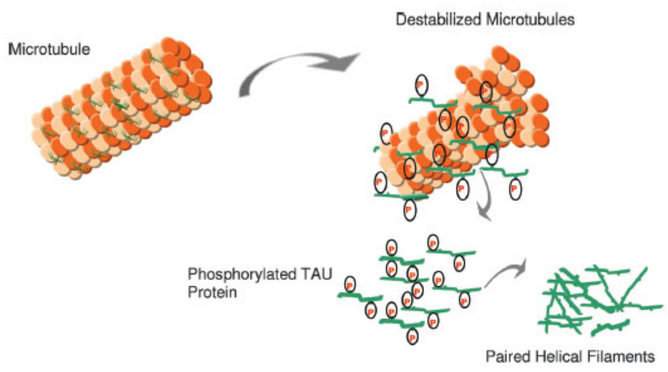


Figure 1 : Tau protein ivolvment in neuronal death

Alzheimer Disease and other tauopathies are linked to the accumulation of aberrant tau protein deposits in neurons. The way how tau forms soluble oligomers, and subsequently paired helical filaments (PHFs) and neurofibrillary tangles is still unclear. Hyperphosphorylation of tau at specific sites may trigger its oligomerisation and consecutive aggregation. Small molecules, such as leucomethylthioninium bismethanesulfonate (LMTM) are thought to prevent aggregation. The present study aimed at monitoring tau oligomer formation after acute treatment with LMTM. Two complementary techniques, Western blot (WB) and mass spectrometry (MS), were compared, analysing samples from wild-type (WT) and transgenic mice expressing human tau harboring the P301S mutation that induces aggregation.

MATERIALS AND METHODS

- WT mice n=9; WT LMTM treated mice n=9 ; P301S mice n= 10 ; P301S LMTM treated mice : n=9 ;
- SDS Page gel were performed on hippocampus brain mouse lysates
- WB analysis was made using K9JA antibody raised against amino acid sequence 224 – 441 of tau (half sequence)
- MS sample preparation consisted in an on gel-digestion protocol (denaturation with DTT; alkylation of cysteine residues with IAA; trypsin digestion), HLB SPE is used to remove salt and to concentrate sample.
- Several proteotypic and specific peptide per species (human/mouse) are selected for SRM analyses.

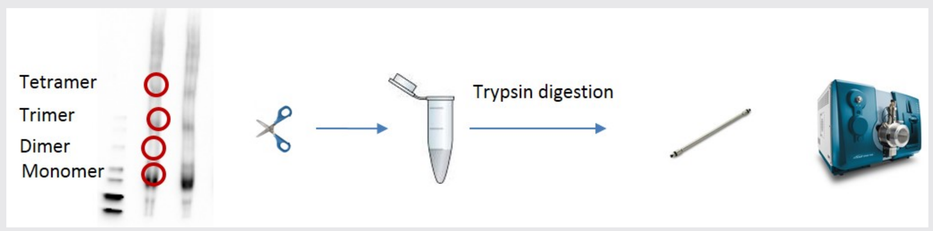
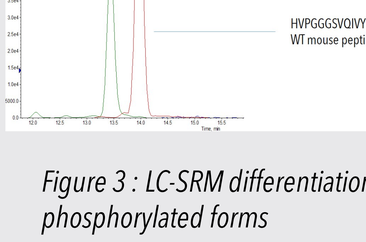


Figure 1: In-gel-digestion process used to observed different oligomer forms



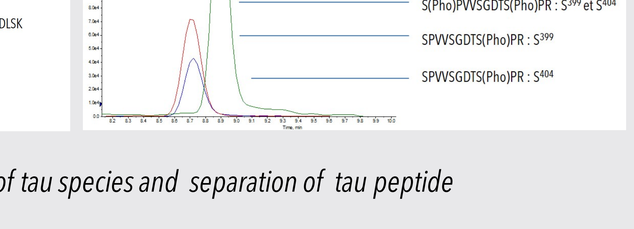


Figure 2: western blot technic versus ions specificity selection in SRM

- Using the high specificity capacities of LC-SRM analysis, we can discriminate species based on a single amino-acid sequence difference and differentiate close phosphorylation sites.

Figure 3 : LC-SRM differentiation of tau species and separation of tau peptide phosphorylated forms

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RESULTS

Hippocampal samples from treated or untreated mice revealed tau different oligoforms. MS specific analysis against WB, allows characterization of animal models: analyzing murine or human tau using MS, we confirmed that the protein overexpressed by P301S mice (8-fold increase compared to WT) reflects only human tau.

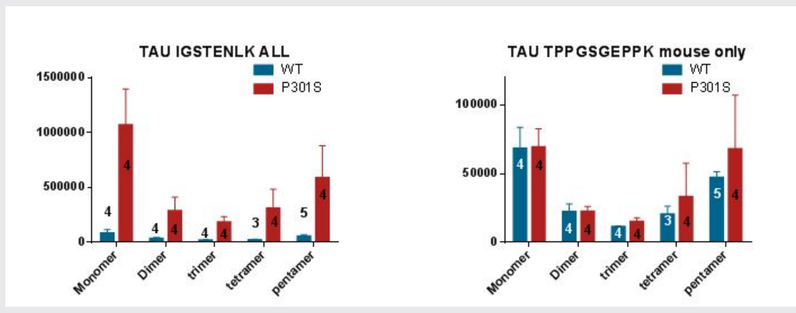

Figure 4: TAU quantity in WT and P301S depending on peptides used : common peptide IGSTENLK (Left) or murine peptide TPPGSGEPPK (right)

After acute LMTM treatment, WB did not reveal any effect on oligomer formation in either wild-type or P301S mice. Figure 5 shows a 2-fold increase in the monomer/oligomer ratio after LMTM treatment of WT mice. No significant change in this ratio was observed in LMTM-treated P301S mice.

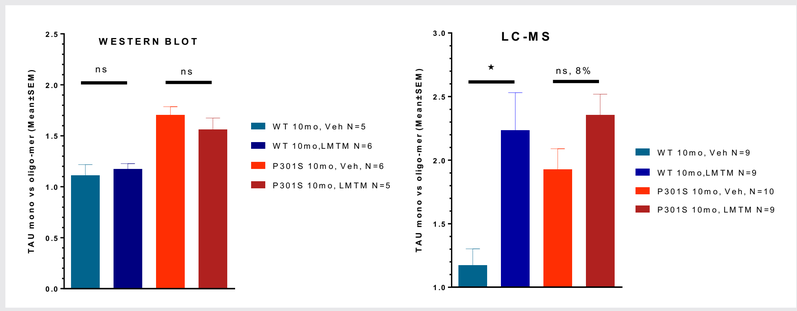

Figure 5: ratio monomer vs oligomer in WB (Left) and mass spectrometry (right)

Looking for specific tau phosphorylation sites, MS revealed an increase in pS404 (but not in other phosphorylation site as pS214-T217 and pS199-S202) in monomers of hippocampal samples from LMTM-treated P301S mice.

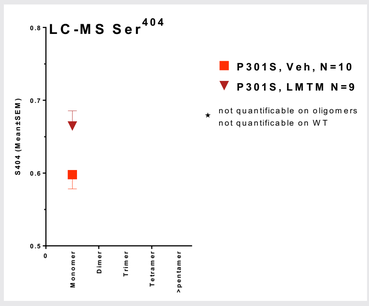

Figure 6: Phosphorylation of TAU S404 observe on P301S mice with or wituout LMTL treatment

CONCLUSION

MS specificity allows characterisation of animal models and could be used to show that P301S mouse was an acceptable model to evaluate human treatment effect. Complementary analyses (WB and MS), show absolute quantification using mass spectrometry can reveal changes in tau oligomer formation after acute treatment with LMTM. These results can be obtained thanks to the high dynamic range of the LC-MS technology.

Moreover, mass spectrometry method allows a selective phosphorylation site detection and bring more accurate information than WB on quantitation of specific phosphorylation sites. The S404 phosphorylation site is over phosphorylated with treatment and thus, this phosphorylation site could participate in the microtubule stabilization improvement or the reduction of TAU oligomer formation.