TAU PHOSPHORYLATION INCLUDING RESIDUE THR217 AND TAU AGGREGATION IN DIFFERENT IN VITRO MODELS

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BACKGROUND

Recent studies highlight phosphorylated tau at residue threonine 217 (pT217) as a new promising plasma biomarker for pathological changes implicated in Alzheimer's disease (AD) and therefore it also gained attention as possible target in AD therapeutics. To provide platforms to screen for pT217-related drugs and assess pT217 implications in AD we tested the presence of pT217 and different other pTau sites in two established *in vitro* models.

MATERIAL & METHODS

SH-SY5Y cells overexpressing hTau441-P301L as well as primary cortical neurons isolated from PS19 embryos were cultivated and treated with different compounds, like kinase inhibitor (CHIR99021), autophagy modulator (rapamycin) or anti-aggregatory agents (Anle138) for 4 h, 24 h and 48 h.
The levels of total tau as well as tau phosphorylated at T217, T231 or S396 were evaluated by immunocytochemistry (ICC) and normalized to total tau signal assessed with Tau13 antibody. Tau aggregation was tested for some conditions using either an image-based approach, counting intracellular spots or via a HTRF-based assay.

RESULTS

SH-SY5Y hTau441-P301L cells

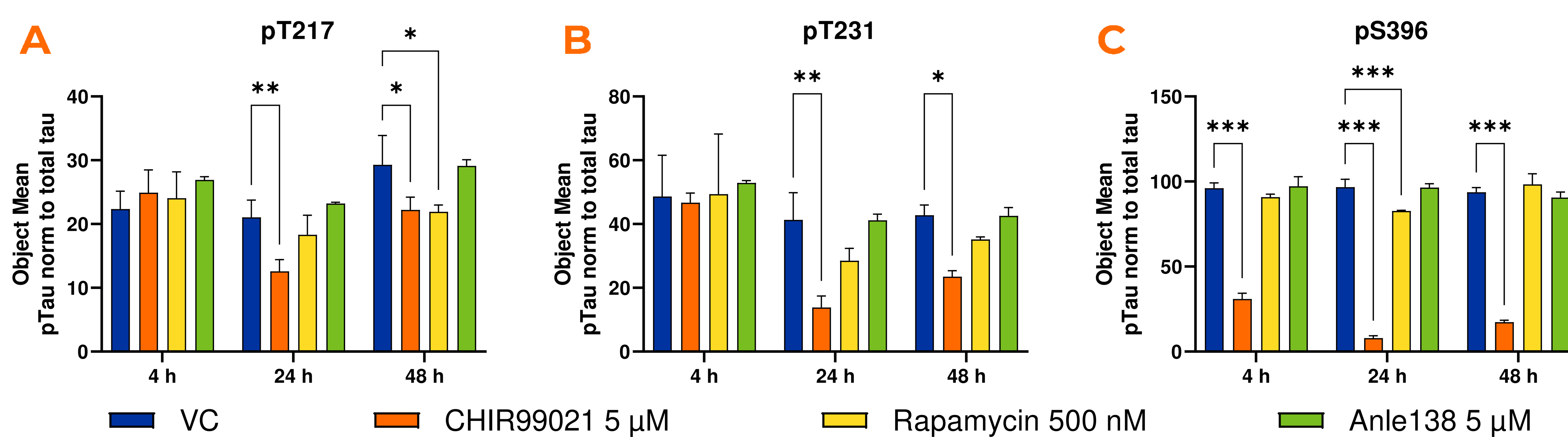
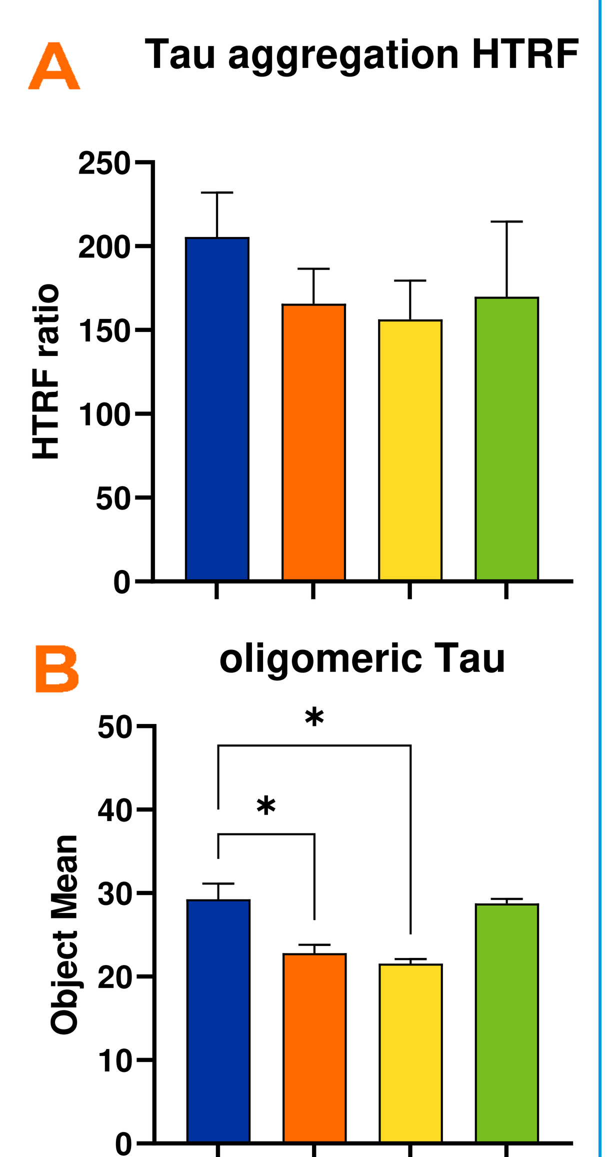


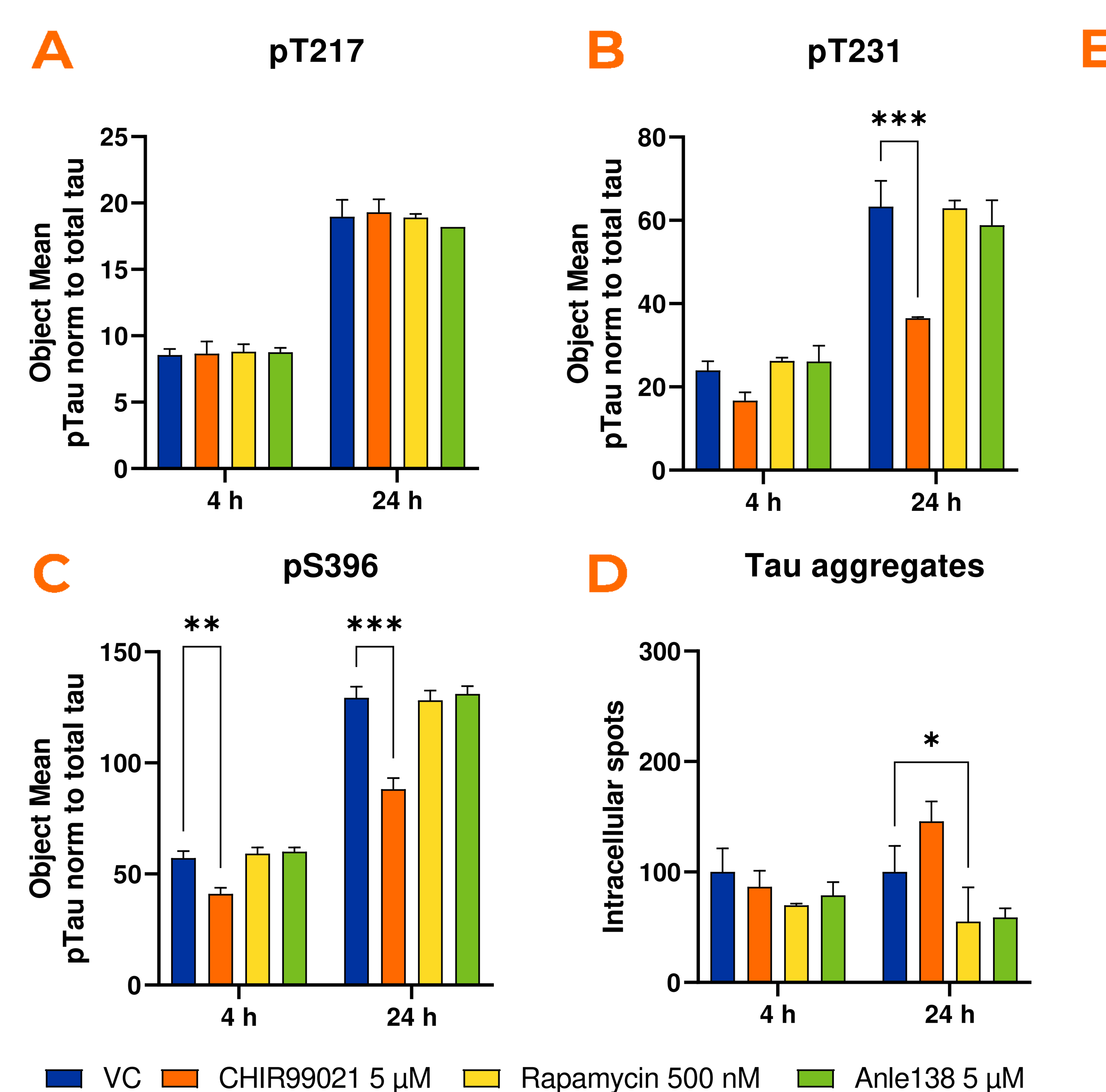
Figure 1: Tau phosphorylation time course in SH-SY5Y hTau441-P301L cells. Tau phosphorylated at (A) pT217, (B) pT231 and (C) pS396 assessed via ICC at three different time points. Data are given as signal intensity mean normalized to total Tau. Treatment with kinase inhibitor CHIR99021 significantly reduced all 3 analyzed ptau sites at different time points. Rapamycin reduced phosphorylation at residue T217 after 48 h and pS396 after 24 h. Anle138 had no significant impact on tau phosphorylation, as expected. Two-way ANOVA followed by Dunnett's *post hoc* test compared to vehicle control (VC). Mean ± SEM. *p<0.05, **p<0.01 and ***p<0.001. n= 3-6 per group.

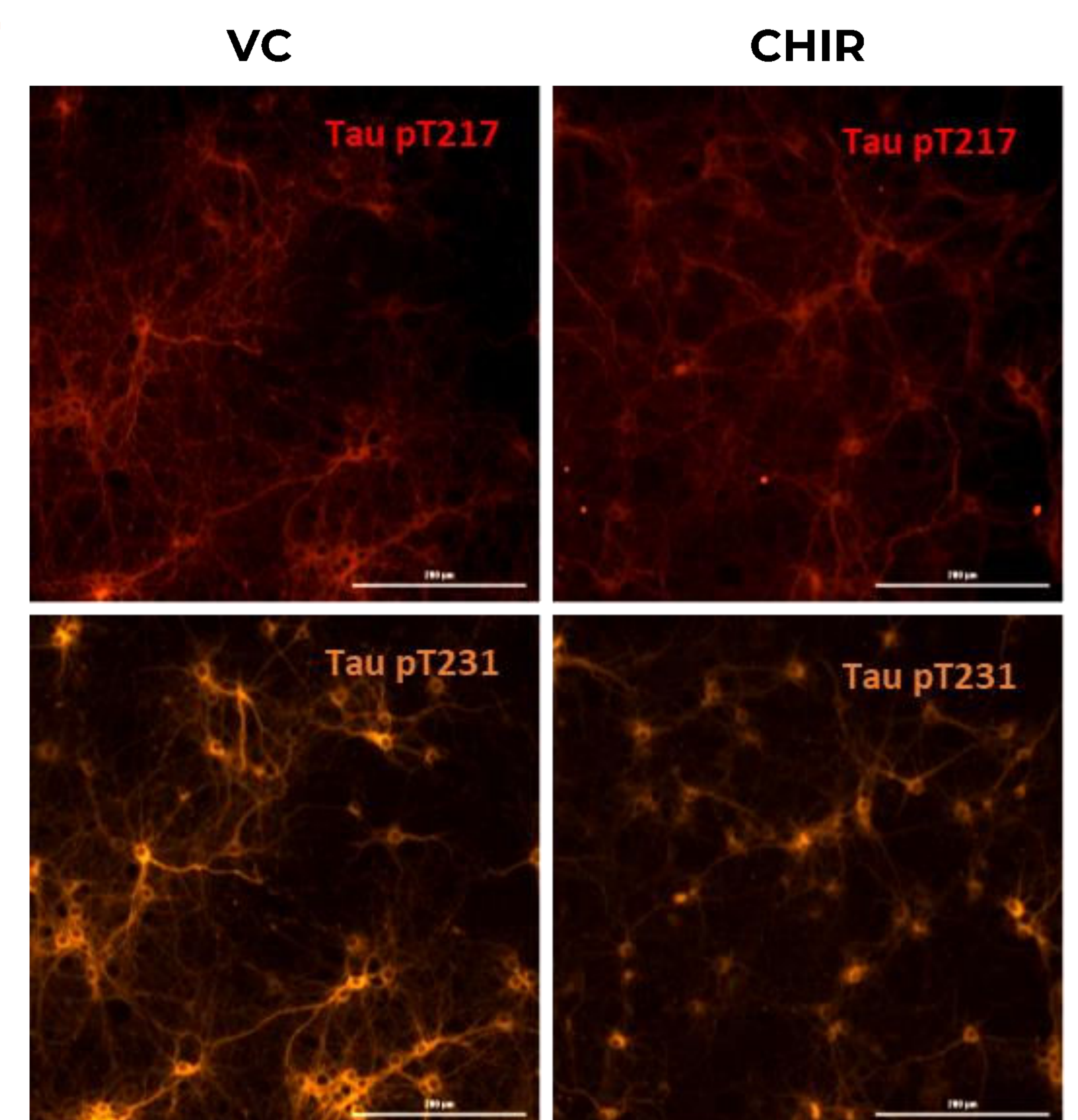
Figure 2: Tau aggregation in SH-SY5Y hTau441-P301L cells after 48 h treatment. Tau aggregation was assessed via (A) HTRF-based assay or (B) ICC, using a tau oligomer-specific antibody. One-way ANOVA followed by Dunnett's *post hoc* test compared to vehicle control (VC). Mean ± SEM. *p<0.05, n= 3-6 per group.



Primary embryonic cortical neurons from PS19 mice

Figure 3: Tau phosphorylation and aggregation time course in primary cortical neurons from PS19 mice. Tau phosphorylated at (A) pT217, (B) pT231 and (C) pS396 as well as tau aggregates assessed via ICC at two different time points. Data are given as signal intensity mean normalized to total tau (A-C) or number of tau-positive intracellular spots (D). While tau phosphorylation at residue T217 was not affected at any condition in this model, treatment with kinase inhibitor CHIR99021 significantly reduced the other analyzed ptau sites at different time points. Rapamycin significantly reduced intracellular tau spots after 24 h with certain variability. Two-way ANOVA followed by Dunnett's *post hoc* test compared to vehicle control (VC). Mean ± SEM. *p<0.05, **p<0.01 and ***p<0.001. n= 2-6 per group. (E) Representative ICC images of pT217 (red) and pT231 (orange) staining after 24 h VC (left) and CHIR99021 (right) treatment. Scale bar: 200 µM.





CONCLUSION

In vitro methods to screen for the activity of compounds are of high relevance for the early stages of drug development. The here presented assays are developed as complementary tools to our transgenic and induced *in vivo* tauopathy models. In both presented models, SH-SY5Y cells overexpressing hTau441-P301L as well as primary cortical neurons isolated from PS19 embryos, the presence of pT217 phosphorylation was shown. However only in SH-SY5Y hTau441-P301L cells, this site could be modulated with established compounds. Additionally, tau aggregation was assessed via different approaches and reduction could be observed in both model systems by treatment with rapamycin, a well-described autophagy modulator.

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