The Impact of Prolyl Oligopeptidase (PREP) Inhibitors on alpha-Synuclein Aggregation

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In 1998, Spillantini et al. showed that misfolded and aggregated alpha-synuclein (aSyn) is the major component of Lewy bodies, the histopathological hallmarks of Parkinson's disease (PD).[1] Thereafter, aSyn oligomers and fibrils have been identified as toxic species and dopaminergic neurons of *substantia nigra* are particularly vulnerable for aSyn toxicity. This has made aSyn as an interesting drug target to have a disease-modifying therapy against PD.[2] One approach has been to target factors that induce aSyn aggregation, and one such target is a serine protease, prolyl oligopeptidase (PREP).

Small-molecule PREP inhibitors were extensively studied as memory enhancers via increased brain neuropeptide levels starting from early 1980s. However, although some positive effects were seen in experimental memory models, their impact on neuropeptide levels remained controversial.[3] In 2008, Brandt et al. showed that PREP accelerates the aggregation process of aSyn and this can be blocked by PREP inhibitors.[4] Thereafter, we have studied the impact of PREP and its inhibition on aSyn aggregation further, and shown that PREP directly interacts with aSyn to increase its dimerization and the presence of PREP leads to formation of soluble aSyn oligomers *in vivo*.[5,6] Moreover, our lab has shown that even a short-term inhibition of PREP by our reference compound, KYP-2047;

1. Modifies the interaction between PREP and aSyn that leads to decreased aSyn dimerization.[6]
2. Reduces stress-induced aSyn aggregates and increases cell viability in aSyn overexpressing cellular and mice models.[7-10]
3. Enhances degradation of aSyn oligomers by enhancing macroautophagy.[5,9]
4. Can restore the behavioural deficit caused by aSyn in a mouse model.[11]

When we studied the interaction between PREP and aSyn, we noticed that also catalytically inactive PREP (S554A PREP) forms an interaction and enhances the aSyn dimerization, suggesting that enzymatic activity of PREP is not required for aSyn aggregation process. Therefore, we wanted to test small-molecule PREP inhibitors with different structures (Kuopio Yliopisto Products, KYP-compounds) in aSyn dimerization and autophagy assays. We observed that IC50 value of given inhibitor did not correlate neither with reduction in aSyn dimerization or enhanced autophagy. Based on these findings, we suggest that the impact of PREP on aSyn aggregation and autophagy is related to conformational changes of PREP, and different PREP inhibitor compounds binding differently to PREP cause altered conformational changes on PREP.

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