New Drugs for Treatment of Parkinson’s Disease

The pathology of Parkinson’s disease is not well understood but it is clearly linked to the misfolding and aggregation of the protein \( \alpha \)-synuclein in neuronal cells. The inventors through their knowledge of \( \alpha \)-synuclein druggability have developed new drugs that bind \( \alpha \)-synuclein with high affinity and prevent protein aggregation making them promising leads to treat or slow the progression of Parkinson’s disease.

**Parkinson’s disease**: An estimated seven to ten million people worldwide are living with Parkinson’s disease (PD), with associated costs in the US of $25 billion per year. In Saskatchewan, the incidence of PD is over 1% in adults over 65 years of age with evidence that exposure to agricultural chemicals increases the risk of disease. The characteristic pathogenic feature of PD is the aggregation of \( \alpha \)-synuclein into fibrils known as Lewy bodies. \( \alpha \)-Synuclein is a natively unfolded protein whose structure is extremely sensitive to its environment. Misfolding of \( \alpha \)-synuclein eventually leads to insoluble fibrils and inclusion bodies. The cause of neuronal cell death in PD is not well understood and it has been suggested that increasing the activity of the misfolded protein clearance machinery or decreasing the rate of mitophagy might provide suitable therapies. However, since the misfolding and aggregation of \( \alpha \)-synuclein appears to be the key toxic event, an emerging therapeutic target is disruption of the initial step of the misfolding pathway.

**Drug target**: It is postulated that drugs which bind to \( \alpha \)-synuclein and cause the protein to adopt a loop conformation will prevent its aggregation and slow or stop the progression of Parkinson’s disease. Drs. Lee and Krol have demonstrated that drugs such as caffeine, nicotine and 1-aminoindan bind weakly to \( \alpha \)-synuclein, causing this loop conformation which prevents aggregation (Figure 1). Epidemiological evidence also shows these drugs are neuroprotective and lower the incidence of Parkinson’s disease. Using this knowledge, Drs. Lee and Krol are linking neuroprotective fragments of these drugs to produce novel compounds which are more effective in binding to \( \alpha \)-synuclein, preventing misfolding and aggregation which could potentially be used to treat and slow the development Parkinson’s disease.

**Technology**: Dr. Krol has used fragment based drug design to create a library of compounds optimized to disrupt \( \alpha \)-synuclein misfolding. As \( \alpha \)-synuclein is an intrinsically disordered protein, no crystal structure is available. Dr. Lee is currently utilizing 3 assays to determine the potential efficacy of our compounds:

- **Nanopore analysis**: This technique describes single molecule interactions between our compounds and \( \alpha \)-synuclein and demonstrates whether the interaction is likely to favour or prevent aggregation of \( \alpha \)-synuclein
- **Isothermal Titrination Calorimetry**: This technique determines the binding affinity of our compounds to \( \alpha \)-synuclein
- **YTH 2 W303 \( \alpha \)-GFP**: This is an \( \alpha \)-synuclein expressing yeast cell line which overexpresses \( \alpha \)-synuclein in response to 5 mM galactose resulting in cell death. Similar cell lines have been identified as providing a druggable target and this system is being used to determine the ability of our compounds to rescue cells from \( \alpha \)-synuclein-induced toxicity

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**Progress**: Out of a library of 20 compounds designed and tested, two, KL1 and KL2 bind to \( \alpha \)-synuclein with an affinity greater than \( K_d \) \( 10^8 \) M\(^{-1}\). That is more than 285 times higher affinity than caffeine and 200 times higher than nicotine. These compounds have been assessed for their ability to rescue the YTH 2 W303 \( \alpha \)-GFP yeast cell line from cell death (treated with 5 mM galactose to induce overexpression of \( \alpha \)-synuclein) over time. As can be seen in figure 2(A), at 1.0 \( \mu \)M both KL1 and KL2, rescue cells at 24 h, however at 0.1 \( \mu \)M the rescuing effect of KL2 is minimal, only KL1 maintains the level of protective effect seen at 1.0 \( \mu \)M. Some rescue by KL1 is still observed at 10 \( \mu \)M (Figure 2(C)).

**Future work**: Use of PD models and PET studies to assess tissue distribution

- Development of radiolabeled probes, for potential early diagnosis
- Further structural optimization of leads for \( \alpha \)-synuclein amyloid selectivity

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Figure 1. As shown in Figure 1A, the conformation of Alpha-synuclein can be investigated by nanopore analysis. In the same way, nanopore analysis can be used to study drug binding to the protein since drug-induced conformational changes can be readily detected. The best strategy for treating PD or preventing disease progression would appear to be prevention of the formation of the early misfolded intermediates. (Figure 1B) Research from several groups strongly suggests that it is the early misfolded structures which are most toxic to the cell and thus drugs which only prevent fibril formation may not be helpful.

Figure 2. Optical density measured at 600nm at 2 h intervals is a measure of cell growth. Paraquat and amphetamine were used as positive controls for cell death and are not included in panel B and C.