Structural study of P-glycoprotein-mediated transport of Aβ across the blood-brain barrier in Alzheimer's disease

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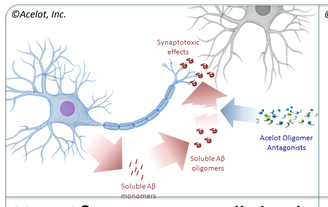
INTRODUCTION

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that affects over 35 million people and leads to a severe loss of mental function.[1]

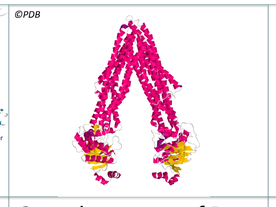
The cause of AD is unclear. However, research has revealed that the disease is characterized by the accumulation of amyloid beta (Aβ), a protein that can form toxic oligomers.[1] While the cause of the accumulation of Aβ is not known, the "amyloid hypothesis" suggests it is due to an imbalance between Aβ production and clearance.[2] Further evidence suggests that transport across the blood-brain barrier may have an important role in the observed reduced clearance of Aβ.[3]

Recently, it has been suggested that P-glycoprotein (Pgp) is involved in the clearance of Aβ from the brain.[4] Pgp is a membrane protein that has a protective role in the body, as it is expressed at barriers like the intestine, and also effluxes many xenobiotics.[4] Pgp is highly expressed at the luminal (blood) side of the blood-brain barrier.[5] There, it extrudes substances from the brain to the blood, including toxins.[5] In this way, Pgp plays an important role in protecting the brain.

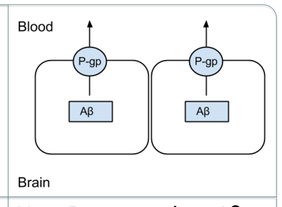
Studies have provided evidence that Pgp may clear Aβ from the brain to the blood.[4] One study showed lower Aβ accumulation with higher Pgp expression.[6] Available data suggests that Pgp is involved in the clearance of Aβ, and thus may be a significant factor in AD.



How Aβ may cause cell death



Crystal structure of Pgp



How Pgp may clear Aβ

METHODS

GROWTH

Express mouse Pgp in P. pastoris

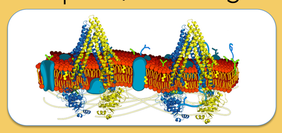
Grow P. pastoris

Induce Pgp expression with methanol

Harvesting

PURIFICATION

Disruption, Centrifugation



Detergent solubilization

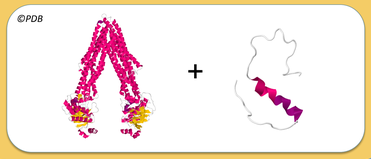
Ni affinity chromatography

Size exclusion chromatography

Protein concentration

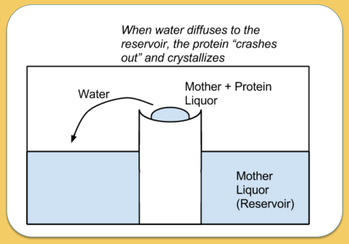
CRYSTALLIZATION

Pgp incubated with Aβ



Reductive methylation

Set crystallization trays with mother liquor and protein



RESULTS

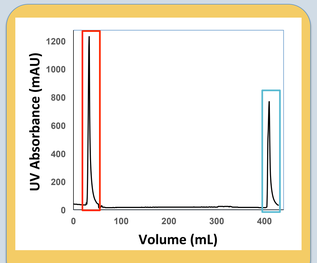


Figure A: UV absorbance of material running through Ni affinity chromatography.

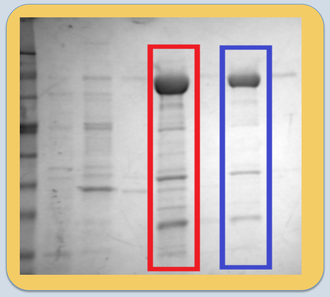


Figure B: Results from SDS-PAGE of protein after Ni purification.

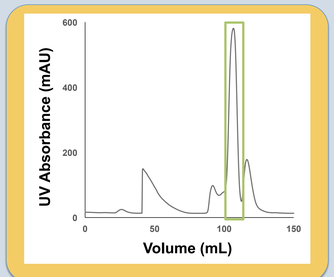


Figure C: UV absorbance of material running through size exclusion chromatography.

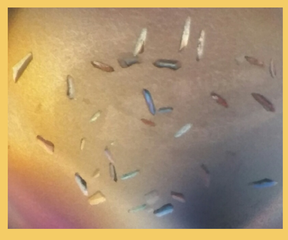


Figure D: Crystals of Pgp grown after incubation with Aβ.

The pictures above demonstrate results showing that we were able to successfully purify Pgp and form crystals of Pgp in trials where Pgp was co-treated with Aβ. The crystals will be tested for presence of Aβ and, if positive, used for structural elucidation.

CONCLUSIONS

We were able to successfully express mouse P-glycoprotein in yeast and purify the protein using various techniques. We also obtained crystals in conditions where Pgp was incubated with Aβ. We will verify if the crystals that appeared in these conditions are Pgp-Aβ co-crystals. If Aβ is present, we will obtain x-ray diffraction data towards generating a structural model of the Aβ-Pgp interaction. The structure would show how Pgp interacts with Aβ at the atomic level. Furthermore, this structure would show if any conformational changes occur, and with which residues in the drug-binding pocket Aβ interacts. The ultimate goal is using the co-crystal structure in order to further characterize Pgp-mediated transport of Aβ, which is a promising target in Alzheimer's disease.

REFERENCES

1. Querfurth, Henry W., et al. "Alzheimer's Disease." *N Engl J Med* 362.4 (2010): 329-44.
2. Hardy, John, et. al. "The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics." *Science* 297.5580 (2002): 353-56.
3. Deane, Rashid, et. al. "Role of the Blood-Brain Barrier in the Pathogenesis of Alzheimers Disease." *Current Alzheimer Research* 4.2 (2007): 191-97.
4. Vogelgesang, Silke, et. al. "The Role of the ATP-Binding Cassette Transporter P-Glycoprotein in the Transport of β -Amyloid Across the Blood-Brain Barrier." *Current Pharmaceutical Design* 17.26 (2011): 2778-786.
5. De Lange, Elizaeth C. "Potential Role of ABC Transporters as a Detoxification System at the Blood–CSF Barrier." *Advanced Drug Delivery Reviews* 56.12 (2004): 1793-809.
6. Vogelgesang, Silke. "Deposition of Alzheimer's Beta-amyloid Is Inversely Correlated with P-glycoprotein Expression in the Brains of Elderly Non-demented Humans." *Pharmacogenetics* 12.7 (2002): 535-41.