Glycan Derivatization to Improve IgG Permeability Through the Blood Brain Barrier to Effectively Treat Alzheimer's

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ABSTRACT

Alzheimer's is a neurodegenerative disease that has affected more than 6 million families. Although there have been many attempts to find a cure for the disease, none have been successful in either the prevention or curing of Alzheimer's. The biggest obstacle that makes Alzheimer's so hard to treat is the inability to transport medicine through the blood-brain barrier (BBB). Prior research indicates that human antibodies, IgG, are not able to cross this blood-brain barrier successfully. 4G8, however, has been reported to have a reduced efflux, meaning decreased 'traffic out of the brain' with a non-impacted influx, referring to the 'traffic' into the brain, with the treatment of neuraminidase. 4G8 is an IgG antibody that has a sialylated Fab Glycan that specifically binds to and recognizes amyloid plaques in the brain; these plaques are the driving factor in causing Alzheimer's. In order to increase the permeability of IgG through the BBB, we will use glycosylation, the addition of a sugar molecule, to make these antibodies homogenous to 4G8 by removing IgG's sialic acid group and replacing it with that of 4G8. We are hoping that making the Fab Glycan of IgG homogeneous to 4G8 should allow for a more effective means of treating Alzheimer's. Although we were able to glycosylate the antibodies successfully, we were not yet able to determine if this would be an effective method to promote the crossing of the blood-brain barrier.

INTRODUCTION:

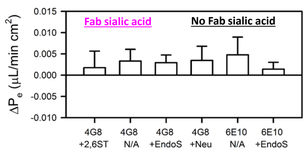
Alzheimer's is a neurodegenerative disease that has unfortunately impacted more than 6 million families in the United States, alone[1]. Alzheimer's is involved in the disruption of neurons that are vital for communication, the repairing of the body, as well as metabolism[2]. These neurons are disrupted by the formation of amyloid plaques which are formed by breaking down the amyloid precursor protein[2]. Previous studies have shown that IgG is an effective means of trying to target these proteins, but the blood-brain barrier (BBB) poses a problem. Due to the low permeability of the BBB, this presents an obstacle in trying to get a sufficient amount of IgG through in order to target the proteins before they become plaques. It is through this project that we attempt to find a solution that will allow IgG to be able to travel through the BBB more effectively.

BBB PERMEABILITY EXPERIMENTS

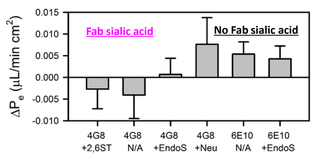
- Wanted to test BBB permeability in vitro
- Tested whether Fab sialylation impacted this permeability (efflux vs. influx)
- Fab sialylation is when you introduce a Fab group to a molecule.
- This is being done in hopes of it influencing the permeability of antibodies through the BBB

We compared the influx and efflux differences both with the presence of Fab sialic acid and the absence of Fab sialic acid. The results are shown here to the right:

Influx Results: No Significant Difference

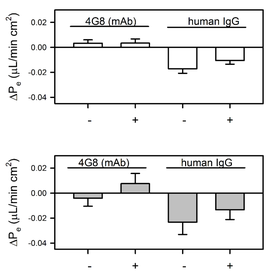


Efflux Results: Efflux Reduced With Fab Sialic Acid



Further Implications:

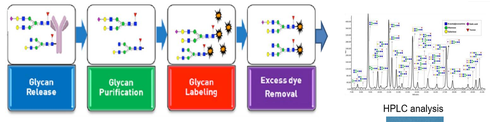
- Why does this matter?
- Correlation to antibodies (and the IgG mentioned in the Introduction)
- So, what now?



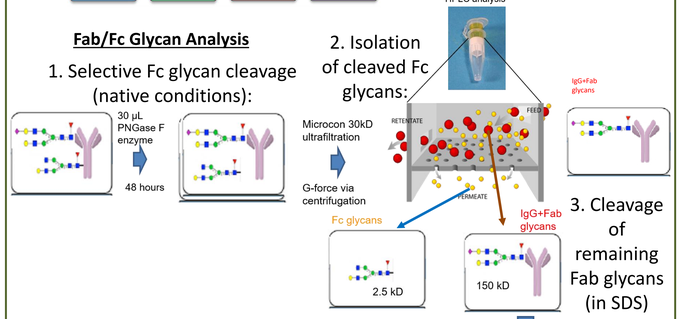
Ultimate Goal: Take the sialic acid group off the 4G8 antibody and swap it with the sialic acid group that is originally on the IgG antibody.

Methods and Materials

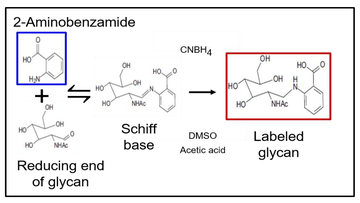
Glycan Analysis- General Process

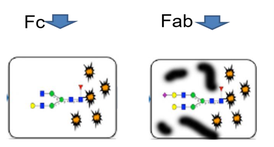


Fab/Fc Glycan Analysis

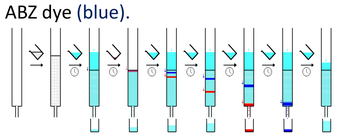


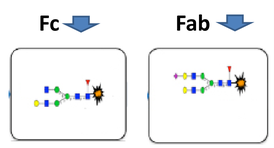
4. Fc and Fab glycans are lyophilized and labeled via reductive amination using 2-aminobenzamide and $CNBH_4$ reductant:



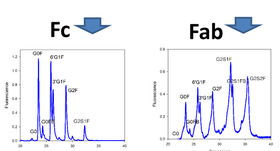


5. Labeled glycans were purified with size exclusion chromatography. Glycans (red) eluted earlier than free ABZ dye (blue).





6. HPLC profile of glycans with polar glycan column. Glycans eluted between 20-40% ammonium formate pH 4.5 in acetonitrile. Measured with ABZ fluorescence.



RESULTS/TRIBUTE

Unfortunately, due to Dr. Finke's unfortunate passing, my lab group and I were unable to obtain our results. In lieu of the results, I have decided to make this final section a dedication to Dr. John M. Finke, a soul who has touched many lives and will continue to do so for as long as people will remember him. I had the blessing to have been Dr. Finke's student for three quarters in a row. Because of this, he and I grew very close, and in a way, he was like my college dad. Dr. Finke had this amazing superpower at being able to turn your worst days into some of the best ones with his corny dad jokes and amazing advice for how to turn your day around. I have never enjoyed a class as much as I did in my capstone class I had with Dr. Finke, not only because I was very excited about our research, but because every single day of that class was filled with laughter, jokes, and easily some of my best memories in college. I will be forever grateful for all the wisdom Dr. Finke has bestowed upon me, and I know that this is a feeling that many students share with me as well.

Despite these unfortunate circumstances and some of the most harrowing experiences both I and other colleagues and professors alike have had to face, I would also like to give recognition to Dr. Jutta Heller and Dr. Joyce Dinglasan-Panlilio. Although I know this effort has stretched beyond these two professors, I would like to separately acknowledge these two professors here. Dr. Joyce serves as the Division Chair of SAM Division, as many of you know, so a lot of weight has fallen onto her shoulders to ensure that all the students impacted by Dr. Finke's unfortunate loss is taken care of. As for Dr. Jutta Heller, she is the professor who had to take over Dr. Finke's class after his passing. The task alone is no easy one to take, but Dr. Jutta has done an amazing job at helping all of us, especially me, with both our grieving of Dr. Finke, as well as trying to navigate what to do next, especially when it came to our research. She has done an amazing job at keeping all of us on our feet, and for that I have her to forever thank for making these hard times so much easier on us.

REFERENCES

[1]Mayo Clinic. 2023 Feb 2. Alzheimer's disease - symptoms and causes. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447.

[2]National Institute on Aging. 2018 May 16. What Happens to the Brain in Alzheimer's Disease? National Institute on Aging. https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease.