The Role of α-synuclein in Heparin-Induced Platelet Activation and Aggregation

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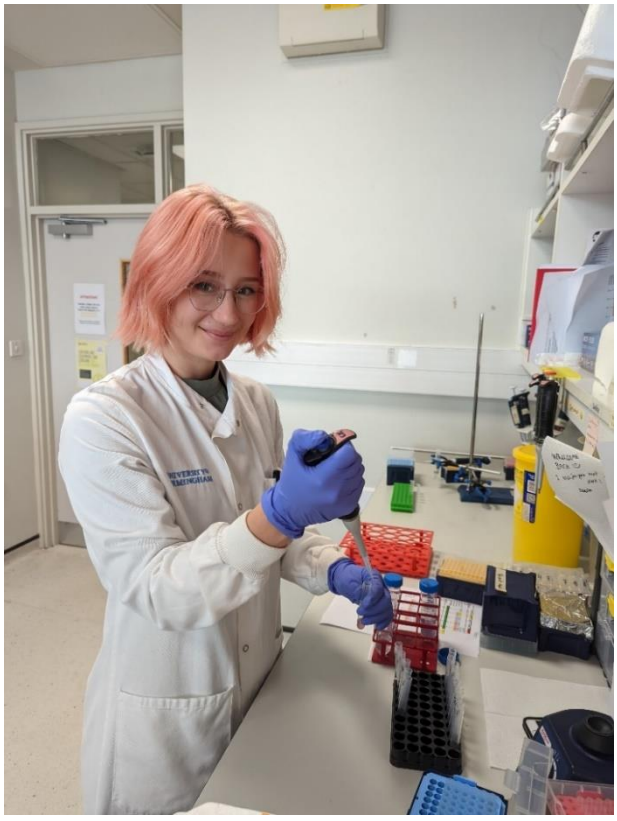
Lay Summary

Parkinson's Disease (PD) is a neurodegenerative disorder involving the nerve cells of the brain, causing uncontrollable movements such as tremors. For an unknown reason, rising evidence suggests people with PD could be at greater risk of complications related to blood clotting, e.g. stroke. These are potentially fatal complications and can worsen the Parkinsonian symptoms. Our project involved investigating the potential mechanism of this high platelet activation that potentially leads to blood clots. We looked at the effect of α-synuclein - a protein that gathers in the brain of Parkinson's patients and causes it to degenerate. We treated blood platelets with α-synuclein in combination with heparin – a glycosaminoglycan released from immune cells called mast cells, which can be found in connective tissue but also around blood vessels and in the brain. We measured whether the two of these together lead to activation of platelets and increased the risk of blood clot formation.

Me in the Lab



Project Aim

To test the platelet response to different doses of α-synuclein in combination with heparin and to shed light upon the underlying signalling mechanism.

Methods

Every week, blood samples from different healthy donors were analysed using three main techniques to investigate the effect of α-synuclein and heparin on platelets. We centrifuged and washed platelets from whole blood and analysed them on a light transmission aggregometer using different doses of α-synuclein alone and in combination with one low dose of heparin to study whether the platelets aggregated. We also performed Western blots to look at Serine473 phosphorylation of Atk which is a key signalling pathway activated by the Platelet Endothelial Aggregation Receptor 1 (PEAR1) in platelets. We also used a pan-tyrosine phosphorylation antibody to look for general tyrosine kinase mediated platelet activation. Whole blood samples were analysed using flow cytometry: antibodies against α-granule marker CD62p and activated integrin αIIbβIII (PAC-1) were used to monitor platelet activation in response to different doses of α-synuclein alone and with heparin. Additionally, we used the mitochondrial membrane marker DilC to investigate if the combination of α-synuclein and heparin could cause formation of pro-coagulant platelets. Later in the project, we tried immunoprecipitation using α-synuclein antibodies to look at the signalling mechanism involved. We also tried expansion microscopy using stains for CD41a, vWF and PEAR1 to look at potential microaggregate formation.

Findings

To investigate the degree of platelet activation in response to α-synuclein and heparin, we used flow cytometry. We found that the number of cells positive for CD62p and PAC-1 increased significantly in the whole blood samples treated with heparin and α-synuclein together compared to those treated with each mono-treatment. The combination treatment increased the percentage CD62p positive cells. However, the major effect was observed on the percentage of PAC-1 positive cells - suggesting that the heparin and α-synuclein combination leads to more integrin αIIbβIII activation than granular secretion in comparison to collagen related peptide and adenosine diphosphate that were used in the positive controls. This shows that α-synuclein and heparin cause synergistic effect on platelet activation. Flow cytometry using DilC was used to investigate the mitochondrial membrane potential ($\Delta\Psi m$) of platelets. We found that there was reduced DilC mean fluorescence in the combined treatment sample compared to the mono-treated samples. This shows a slight disruption of mitochondrial membrane potential and suggests that platelets might become pro-coagulant in the presence of heparin and α-synuclein together. Future investigations of Annexin V expression are required to confirm this result.

We used light transmission aggregometry to measure platelet aggregation in response to different doses of heparin and α-synuclein alone and together. We saw a small increase in light transmission for dual-treated samples, but most donors did not display secondary activation. The low level of

CD62p expression observed in the flow cytometry experiments might explain this result- as secondary activation involves granular release.

To investigate the cell signalling mechanisms, Western blotting and immunoprecipitation were used. Western blots using an Akt-Ser473 phosphorylation antibody showed a small increase in optical density whilst a pan-tyrosine phosphorylation antibody did not show any significant increase in phosphorylation. Upon immunoprecipitation using α-synuclein antibodies, we saw that the combination treatment failed to immunoprecipitate α-synuclein, meaning heparin may interfere with antibody binding to α-synuclein.

In conclusion, we found that heparin and α-synuclein have a synergistic effect on platelet activation. The combination leads to increased integrin αIIbβIII expression, α-granule release, and altering of mitochondrial membrane potential, possibly making the platelets pro-coagulant. Our findings give some insight into the increased thrombosis risk in PD patients, but further details of the exact underlying mechanism are yet to be determined to enable prevention.

What Did I Learn?

This project has been an incredibly valuable learning experience for me. Not only has it helped me learn how to do common experimental methods like western blots and flow cytometry, but also allowed me to understand how and why different methods should be used and how to apply controls – a skill that will aid me greatly in my third-year project and any other future lab work. Due to constant circumstantial changes in the lab, I was able to improve my problem solving and adaptability. This project has allowed me to practice and learn dilution calculations, data handling and statistical analysis. It also helped me develop my personal skills – for example how to plan effectively to prevent mistakes and how to best organise my time in a lab. I think it was important for me to experience an example of a research space and what the general work environment can be like. I also learned the valuable lesson of writing things down, focusing on what I'm doing, and how unpredictable science can be.

The Project's Influence on My Goals

As a result of this project and how engaging I found it, I am now very interested in doing lab-based work for my final year project. It has also piqued my interest for cardiovascular science and so I have chosen 'hypoxia and disease' for my third-year primary module. I was never sure as to whether I wanted to do a PhD but I am now a lot more persuaded to do one after experiencing lab work and discussing the benefits of further education with colleagues. Since the start of my degree, I was most interested pursuing research and development as a career and now, having taken part in this project, it has confirmed my interest in lab work and my career aspirations.