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Cross-seeding of Protegin-1 and Amyloid Beta to inhibit Amyloid Beta aggregation

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Abstract

Amyloid beta (Aβ) is a major component in the progression of degenerative diseases like Alzheimer's disease (AD). To treat AD before it had presented, the effects of antimicrobial peptide protegrin-1 (PG-1) on the aggregation of Aβ was investigated. By cross-seeding to form a PG-1-Aβ complex, it is believed that aggregation of Aβ on the human brain can be inhibited, thus helping to stop the neurodegeneration caused by AD. During this study, the effects of cross-seeding PG-1 with Aβ was investigated in four separate experiments via cross-seeding of PG-1 with: monomeric Aβ, different Aβ seed, with Aβ in a cell, and with Aβ in a bacterial culture. Cross-seeding PG-1 with both monomeric Aβ and performed Aβ showed disaggregation results, lowering the β-sheet content by 8.0-17.7% and 4.8-17.2%, respectively. Additionally, the co-incubation of PG-1 with Aβ-treated SH-SY5Y cells caused an increase in cell viability and decrease in cytotoxicity by 0.2-12.2% and 6.2-10.2%, respectively. Finally, PG-1-Aβ heterocomplexes showed an increase in antimicrobial activity when cultured with four different bacterial strains, with the best case increasing by 33.2-65.8%. These findings show that PG-1 is effective at disrupting the aggregation of Aβ, providing a major step forward in the potential treatment of neurodegenerative diseases.

Executive Summary

Problem statement and introduction

Amyloid beta (Aβ) is a major component in the progression of degenerative diseases like Alzheimer's disease (AD). With millions of people all over the world being affected by AD, it is important to determine a method of halting the disease. Although curing AD after it has progressed is highly unlikely, efforts have been made to identify the underlying cause of the disease in an attempt to halt the progress before it has begun. To treat AD before it has presented, the effects of antimicrobial peptide protegrin-1 (PG-1) on the aggregation of Aβ was investigated. By cross-seeding to form a PG-1-Aβ complex, it is believed that aggregation of Aβ on the human brain can be inhibited, thus helping to stop the neurodegeneration caused by AD. During this study, the effects of cross-seeding PG-1 with Aβ was investigated in four separate experiments via cross-seeding of PG-1 with: monomeric Aβ, different Aβ seed, with Aβ in a cell, and with Aβ in a bacterial culture.

Quantitative results

In a cross-seeding experiment of PG-1 with both monomeric Aβ and different performed Aβ seeds, the disaggregation properties of PG-1 on Aβ were measured using Thioflavin T (ThT) fluorescence and circular dichroism (CD) spectroscopy. For monomeric Aβ, cross-seeding with PG-1 showed a significant decrease in Aβ aggregation and β-sheet structures after completion of the experiment (24h). Using different concentrations of PG-1 (0.1-5μM) cross-seeded with Aβ (20μM), a decrease in Aβ aggregation of 28.7-100% based on ThT fluorescence. From CD spectroscopy, the β-sheet content dropped by 8.0-17.7% when cross-seeding Aβ (20μM) with varying concentrations of PG-1 (0.5-5μM). For different performed Aβ (20μM) seeds, cross-seeding with PG-1 (1-10μM) at different time phases (5h, 15h, 25h, and 36h) showed a decrease

in Aβ aggregation from 25.6-65.5% based on ThT fluorescence. Using CD spectroscopy, the β-sheet content for the different performed Aβ seeds decreased by 4.8-17.2% upon cross-seeding with varying concentrations of PG-1.

To test the ability of PG-1 to decrease the Aβ-induced toxicity in SH-SY5Y neuroblastoma cells, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays were utilized to measure the cell viability and cytotoxicity, respectively, of PG-1-Aβ heterocomplexes (0.5-10μM:20μM). The MTT results showed a sharp increase in cell viability, with the PG-1-Aβ heterocomplexes increasing cell viability by 0.2-12.2%. The LDH results showed a dramatic reduction in cytotoxicity, with PG-1-Aβ heterocomplexes decreasing cell cytotoxicity by 6.2-10.2%.

Finally, PG-1 is a peptide found within a group titled antimicrobial peptides. To determine if PG-1 retained its antimicrobial properties when cross-seeded with Aβ, a bacterial growth assay was formed to test antimicrobial capacity using OD_{600} values. Four bacterial strains were utilized for the experiment: two Gram-negative and two Gram-positive. Upon completion, it was found that the PG-1-Aβ heterocomplexes significantly increased the antimicrobial capacity of the solution. In the best case, the antimicrobial capacity increased by 33.2-65.8% as compared to pure PG-1 and pure Aβ, respectively.

Definite conclusions

PG-1 has strong disaggregation properties on Aβ fibrils. These disaggregation properties, which are found as low as 0.1μM, apply even for Aβ fibrils that have already begun to form β-sheet plaques, aiding in possible treatment of AD. It was also concluded that PG-1 greatly reduces the cytotoxicity of Aβ-induced cell death. As PG-1 is hoped to be used in human brains to stop Aβ aggregation, low cytotoxicity is of the utmost importance to avoid damage to the

patient. Finally, the cross-seeding of PG-1 with Aβ shows promising antimicrobial activity. When treating a patient for AD, bacteria may be introduced into the treatment site. By having a strong antimicrobial capacity for bacteria, even antibiotic-resistant bacteria, the PG-1-Aβ heterocomplexes can greatly reduce the chance of infection in a patient.

Broader implications

Through this project, I feel that I have grown in my knowledge of both engineering and biology. I have learned technical skills working in the lab that will assist me in my career after graduation, including cell cultures, bacterial assay, ThT analysis, CD spectroscopy, pipetting, and data collection. On a personal basis, I have gained confidence working in the lab setting. Before starting this project, I had no experience in a bioengineering type of lab environment. The skills I have learned in this environment will not only help in the lab setting, but also in any future endeavors I begin. As I prepare to enter a career in biopharmaceutical development, I feel prepared to take on any task my employer requests.

Recommendations

Based on the results obtained during this study, it is recommended to continue the study of PG-1 and its effect on Aβ. Continued cell studies are recommended on other cells of the human body, with the hopes of eventually reaching clinical trials. The dosage safety in humans should be investigated before beginning clinical trials. The disaggregation properties of PG-1 on Aβ should also be investigated at lower concentrations of PG-1, based on the safe concentration levels for humans. Overall, the cross-seeding of PG-1 on Aβ to halt the progress of AD is a promising direction that should continue to be focused on.