The β-Hairpins peptides affect Alzheimer's Disease by Regulating the Inflammation of Astroglial and Microglial Cell.

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Abstract:

Alzheimer's disease is the most common type of brain disease now present in the world. Patients with Alzheimer's disease will experience memory loss, trouble with daily tasks, and other cognitive difficulties. In Alzheimer's disease, neuroinflammation and inflammation of astroglial and microglial cells occur. Inflammation of these two cells will damage the neurons in the brain, causing the number of synapses to decrease, which impairs cognitive function. With time, the number of neuroinflammations will increase. β-Hairpins peptides are found in proteins; they play an important role in the self-assembly of peptides and proteins. To further explore the functional mechanism of B-bairn, Adam G Kreutzer and James S Nowick assembled the Aβ-hairpins structure. They found that the synthetic Aβ-hairpins structure could produce toxicity to nerve cells, but the effect on astrocytes and microglia was not further explored. In the present study, we isolate and culture the astroglial cell and microglia cultures from newborn mice's stripped brains. To simulate the beta-hairpin's environment, we will put different concentrations of protein solutions into the cell cultures. To investigate the effects of the designed β-hairpin peptide on astrocytes and microglia, as well as its potential role in modulating the progression of Alzheimer's disease, we conducted experiments to examine the expression of inflammation and cellular viability after the addition of the β-hairpin peptide. This paper only provides theoretical experiment design and possible results about the relationship between the β-Hairpins peptides and the inflammation process of astroglial and microglial cells, which needs further research in the pathology of atherosclerosis. It also provided the possibility that β-hairpin peptides regulate Alzheimer's disease and provided the chance to understand the molecular basis of amyloid diseases.

Keywords: Beta-hairpin peptides, Alzheimer's disease, inflammation, astroglial cell, microglial cell

1. Introduction

Alzheimer's disease is a common dementia that impairs cognitive function, especially memory and thinking. It mainly affects the elderly over the age of 65. At first, patients may only be forgetful, but as time goes on, they may struggle with daily tasks and have trouble recognizing people and places, negatively impacting their lives. The actual cause of Alzheimer's disease is still unclear; current researchers have suggested that it is linked to the increase of amyloid plaques in the brain [1].

AN IMPORTANT FACTOR OF THE DEVELOPMENT OF ALZHEIMER'S DISEASE IS BETA HAIRPIN. B HAIRPIN IS A STRUCTURAL MOTIF WHERE TWO ANTIPARALLEL BETA STRANDS ARE CONNECTED BY A LOOP. B HAIRPIN STRUCTURES ARE SPECIAL BECAUSE THEIR TWISTED SHAPE, HYDROPHOBIC SURFACES, AND EXPOSED HYDROGEN-BONDING EDGES ALLOW THEM TO FORM UNIQUE AND DENSELY PACKED ASSEMBLIES [2]. THE FLEXIBILITY OF PEPTIDES ALLOWS THEM TO SWITCH FREELY BETWEEN A HAIRPIN AND AN EXTENDED CONFORMATION, RESULTING IN AN EXTENSION OF THE B-SHEET. THE CONFORMATION OF PEPTIDE, BETA HAIRPIN, WILL INCREASE THE PROPENSITY TO PEPTIDE AGGREGATION, WHICH CAN LEAD TO THE PRODUCTION OF TOXIC OLIGOMERS AND FIBERS, TOXIC TO NEURONS. RESEARCH IS ONGOING TO BETTER UNDERSTAND THE EXACT TOXIC MECHANISMS OF BETA HAIRPINS AND THEIR FOLDING PATTERNS [3].

ON THE OTHER HAND, ASTROGLIAL AND MICROGLIAL CELLS PLAY CRITICAL ROLES IN OUR BRAIN, SUPPORTING THE FUNCTION OF NEURONAL SYNAPSES AND REMODELING AS WELL AS BEING RESPONSIBLE FOR CLEARING AWAY NERVE DEBRIS, INCLUDING PLAQUES AND TANGLES. ASTROGLIAL IS A TYPE OF GLIAL CELL THAT INCLUDES PHYSICAL AND METABOLIC SUPPORT FOR NEURONS AND ACTS AS AN IMMUNE RESPONSE IN THE INJURED CENTRAL NERVOUS SYSTEM [4]. THE MICROGLIAL IS THE PRIMARY IMMUNE CELL OF THE CENTRAL NERVOUS SYSTEM, WHICH HELPS REGULATE BRAIN DEVELOPMENT, MAINTENANCE OF

NEURONAL NETWORK, AND INJURY REPAIR[5]. During neurodegeneration and ageing, microglia and astrocytes will become activated around the senile plaques in the brain, resulting in morphological changes in the usual neuroinflammatory response induced by Aβ[6]. Microglia will also acquire a unique activation phenotype, causing the number of synapses to decrease, leading to cognitive dysfunction. In Alzheimer's disease, neuronal cell death caused by amyloid beta deposits and Tau tangles will activate microglia and astroglia, causing a neuroinflammatory response, with at least 10 times as many neurons dying as those caused by plaques and tangles[7].

Over the past few years, Adam G. Kreutzer and James S. Nowick's laboratory has used X-ray crystallography to identify undiscovered modes of self-assembly of macrocyclic β-hairpin mimics containing sequences from amyloidogenic peptides and proteins. In their account, they summarize insights into the structures of the elusive amyloid oligomers and may help shed light on the molecular basis of amyloid diseases. Additionally, Adam G. Kreutzer and James S. Nowick's laboratory has already developed macrocyclic β-sheets that are designed to mimic β-hairpins formed by amyloidogenic peptides and proteins[2]

FOR OUR EXPERIMENTS, WE WILL BE ADDING DIFFERENT CONCENTRATIONS OF PEPTIDES TO ASTROGLIAL AND MICROGLIAL CELLS TO MEASURE THE INFLAMMATORY CYTOKINES EXPRESSION AND CELL SURVIVAL RATES, REFLECTING ON THE MECHANISM OF CELLS BY PEPTIDE EFFECTS IN ALZHEIMER'S DISEASE.

2. Methods

To detect whether β-hairpin peptides (PDB 5SUT & PDB 5SUR) affect Alzheimer's disease by regulating microglia and astrocyte inflammation, we learned to use the mice Microglia and astrocytes were isolated for experiments. Additionally, we showed the technology roadmap of the method in Figure 1.

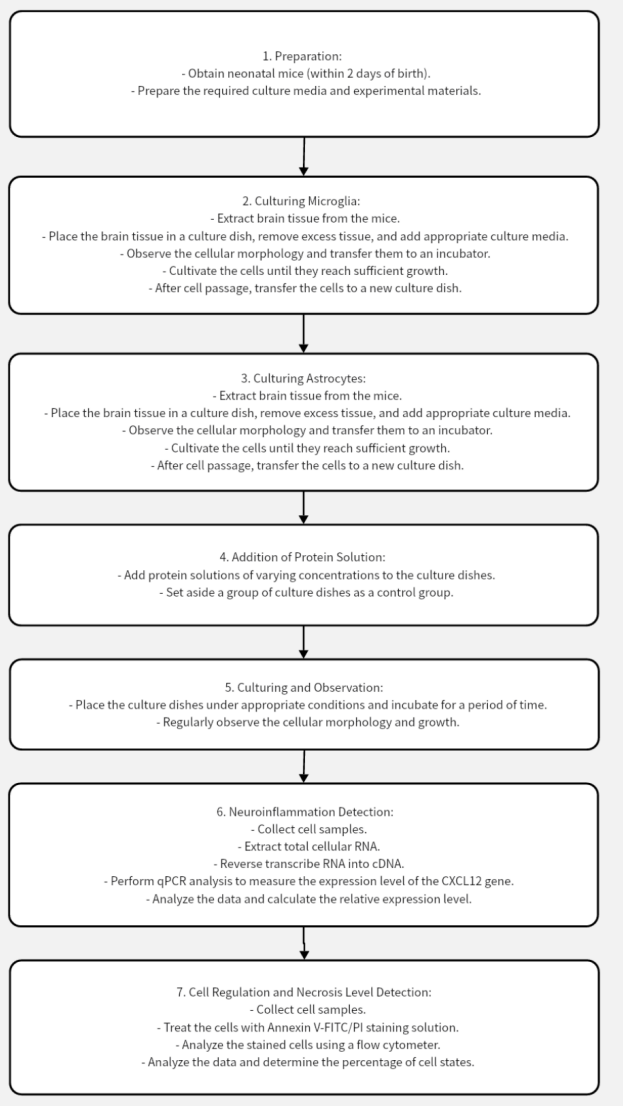

Figure 1 The technology roadmap

Protein solution preparation:

Configure PDB 5SUT and PDB 5SUR with Microglia Medium at 2 μM, 4 μM, 6 μM, and 8 μM concentrations, respectively.

Repeat the above procedure using AstroMACS Medium

experimental manipulation:

Microglia culture:

To make the microglia culture for the investigation to observe its reaction with different concentrations of peptide solution, we will first need to take four 6cm Petri dishes, pour HBSS into them, and place them on an ice tray. We take out four newborn mice within two days of birth, spray them with 75% alcohol, and place them in an ultra-clean workbench. After the mice died, the heads were cut off and rinsed twice in the HBSS buffer. The mice were stripped of their heads and brains with scissors, and the brains were removed with pointed tweezers and

placed in 6-cm Petri dishes. Two were placed in each petri dish, and the olfactory bulb, cerebellum, and the link between the cerebral hemispheres were removed under a dissecting microscope. The stripped brains were placed into new 6cm petri dishes. Dulbecco's modified eagle medium (DMEM), a sterile solution that helps make medium for mammalian cells, was added to the petri dishes. The stripped brain was placed in it, and the brain was cut into paste. Add DMEM to the 100 ml culture flask, put the cells into the encapsulated culture flask, and shake to mix the cells. Label the culture bottle with the name and date. Observe the cell morphology under the microscope and put it into the incubator for culture. Replace the DMEM with Microglia Medium after three days for further optimal growth. The cells will be full-grown and passaged about one week after the medium change. After passaging, put them into 6cm petri dishes, 9 petri dishes are needed for the experiment to compare death cells by their inflammation level.

Astrocytes culture:

To make the astrocytes culture for the investigation to observe its reaction with different concentrations of peptide solution, we will first need to take four 6cm petri dishes and pour Hank's Balanced Salt Solution (HBSS) on an ice tray, take out four neonatal mice within two days of birth, spray them with 75% alcohol, and place them on an ultra-clean bench. After the mice died, the heads were cut off and rinsed twice in the HBSS. The mice were stripped of their heads and brain cases with scissors, and the brains were removed with pointed forceps and placed in 6-cm Petri dishes. Two were placed in each petri dish, and the olfactory bulb, cerebellum, and the link between the cerebral hemispheres were removed under a dissecting microscope. The stripped brains were placed into new 6cm Petri dishes, and AstroMACS Medium, which is used for mouse astrocyte cultivation, was added to the dishes. Place the stripped brain into it and cut the brain into the paste. Add AstroMACS Medium to the 100 ml culture flask, place the cells into the encapsulated culture flask, and shake to mix the cells. Label the culture bottle with the name and date. Observe the cell morphology under a microscope and place it in an incubator. The fluid was changed every three to five days, and the cells were fully grown in about twelve days and then passaged after being fully grown. After passaging, put the cells into 6cm petri dishes. 9 Petri dishes are needed for the experiment to compare death cells by their inflammation level.

According to the previous description, we cultured the microglia and astrocyte cells. To further detect the changes in cellular inflammation after peptide administration, we will add 1ml of different protein solutions into the dishes with cultures and leave one dish for astrocytes and one for microglia as a control group. Wait for three days for later observation. We showed the group division of cells after β-hairpin peptides treatment in Figure 2

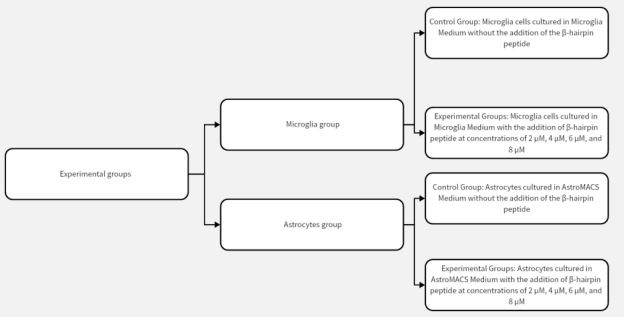

Figure 2 The group division of cells

Determination of neuroinflammation by cxcl12 gets from quantitative polymerase chain reaction:

CXCL12 is closely linked to inflammation through its role in immune cell recruitment, tissue repair, lymphocyte homing, and inflammatory regression. CXCL12-mediated monocyte transmigration into brain perivascular space leads to neuroinflammation and memory deficit in neuropathic pain[8]. Understanding the complex interactions involving CXCL12 in the context of inflammation is critical for improving our understanding of the immune response and developing potential therapies for various inflammatory and autoimmune diseases [9].

Determination of neuroinflammation by another chemokine:

There are also other inflammation factors apart from CXCL12. For instance, there is CCL5 and CXCL1. CCL5 are chemokine proteins associated with chronic inflammation and play an important role in the migration of cells. CCL5 is produced by platelets, macrophages, eosinophils, fibroblasts, endothelium, epithelial and endometrial cells. The variety of cells that express and mediate CCL5 effects implicates this chemokine in multiple biological processes, from pathogen control to enhancement of inflammation in several 'sterile' disorders. CCL5 oligomers interact with the receptors at the three extracellular loops and the amino-terminal portion. The formation of the ligand–receptor complex causes a conformational change in the receptor that activates the subunits Gαi and Gβγ of the G-protein, leading to an increase in levels of cyclic AMP as well as the increase of phagocytic ability, cell survival, and transcription of proinflammatory genes [10]. CXCL1 is a chemoattractant cytokine, which is simply a small peptide. It leads to the infiltration of neutrophils into the sites of inflammatory responses, which contributes to the fight against pathogens or agents that trigger the inflammatory responses, which plays a major role in both the regulation of immune and

inflammatory responses [11].

First, harvest cells. After culturing two kinds of cells, collect the cells using trypsin or other appropriate methods. Next, total RNA extraction. Isolate RNA from the collected microglia and astrocytes using a commercial RNA extraction kit, following the manufacturer's instructions. Then, cDNA Synthesis. Reverse transcribe RNA to cDNA using a reverse transcription kit. This will enable the analysis of gene expression by qPCR. After that, qPCR Analysis. Set up qPCR reactions using primers specific to CXCL12 and a fluorescent probe, such as SYBR Green or TaqMan probes. Run qPCR reactions in a real-time PCR instrument. Finally, Data Analysis. Analyze the qPCR data to determine the relative expression of CXCL12 in microglia and astrocytes compared to a reference gene (housekeeping gene). Calculate the fold change using appropriate statistical methods.

Detection of cellular regulation and necrosis levels with Annexin V-FITC/PI kit:

First, harvest and wash cells. Collect microglia cells and wash them with phosphate-buffered saline (PBS) to remove residual culture media. Next, prepare Cell Suspension. Resuspend the cells in the binding buffer provided in the Annexin V-FITC/PI kit. Then, Staining with Annexin V-FITC and PI. Add Annexin V-FITC and propidium iodide (PI) to the cell suspension. Incubate for the recommended time at room temperature in the dark. After that, flow cytometry analysis. Analyze the stained cells using a flow cytometer. This will allow you to detect and quantify apoptosis and necrosis by measuring Annexin V-FITC and PI fluorescence. Lastly, data interpretation. Use appropriate software to analyze the flow cytometry data and determine the percentage of cells in different states (life, early apoptotic, late apoptotic, necrotic).

The death rates of microglia and astroglial cells could show us the amount of inflammation made by peptides. The higher the death rate, the more inflammation happens. Different concentrations of peptides will cause the destruction of cells or activate cells, which might lead to cell malfunction and change its morphology, which could help increase the expression of inflammation in astroglial and microglial cells. Depending on the concentration level of the protein solution, it will cause different levels of death in astroglial and microglial cells, which could become the driving force of Alzheimer's disease and cause deterioration.

3. Conclusion

To sum up, our experiment has concludedconcluded that depending on the peptide concentration, it would promote cell activation or death. The higher the concentration of peptide, the higher the death rate, which could be the reason for the acceleration of the deterioration of Alzheimer's disease. We intend to explore more about inflammatory cells and the impact of beta hairpins on Alzheimer's disease in the future with more advanced equipment.

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