Researches about important factors for endocytic transport of amyloid beta (Aβ-42) through biomimetic and biological membranes

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Background: Alzheimer's disease (AD) is an age related dementia affecting millions of life globally. Deposition of amyloid beta (Aβ) peptides into extracellular plaques and formation of intracellular neurofibrillary tangles (NFT) are the pathological hallmarks of this neurodegenerative disorder. Aggregation, accumulation, misfolding and cytotoxicity of Aβ peptide are the key events in the pathogenesis of AD. Accumulation of misfolded proteins induces stress to endoplasmic reticulum (ER). This phenomenon is generally known as ER stress which is linked with other cellular responses such as release of calcium ions, inflammation and apoptosis. It has been reported that ER stress may occur by externally added Aβ which would affect the cellular processes. In my study, I have speculated that exogenous Aβ may internalize into the cell via endocytic pathway, then causes stress to the organelle. Thus, I have focused on the factors affecting the peptide transport, there is a critical link between AD and diabetes where oxidative stress, AGEs etc. are the common features between these two ailments. Oxidative stress causes lipid oxidation which generates oxygenated derivatives of cholesterol, known as oxysterols. Recently, oxysterols have been recognized as risk factors for Alzheimer's disease which can be generated by enzymatic oxidation of cholesterol.

Accumulating evidence have suggested that interaction of Aβ with cell membranes has important role in these processes. 7-ketocholesterol (7-KC) and 25-hydroxycholesterol (25-OHC) were the two oxysterols used to substitute the membrane cholesterol level among which latter is produced by reactive oxygen species (ROS) and former gets generated on the enzymatic oxidation to make the transport of cholesterol from brain in form of its derivatives. Notably, toxicity of Aβ depends upon its isoforms and their state of aggregation, thus based on the current understanding, protofibrillar Aβ-42 was used throughout the study.

Aim: In this dissertation, I aimed to investigate the important factors mediating the endocytic transport of Aβ-42. These factors are associated with the risk factors for Alzheimer's disease such as aging, oxidative stress, advanced glycation end products (AGEs), diabetes, genetic or hereditary factors. Thus, in this study, role of oxysterols in AD was emphasized with other factors involved in the endocytic transport of the peptide. Biomimetic and biological membranes possessing different levels of complexities were employed to achieve the aim of this dissertation.

Experimental results and discussion: In chapter 2, after substituting the cholesterol level with 25-OHC in the heterogeneous biomimetic membranes, I have found that the localization of protofibrillar Aβ-42 was influenced in comparison to the membrane without substitution. Predominantly, it occurred in the

liquid-disordered (Ld) phase of lateral compartments of the membrane as shown in the figure 1. Next, I assess the aggregation kinetics of the peptide in the presence and absence of oxysterol. As oxysterols are cytotoxic in nature, thus presumably, they would enhance the toxicity induced by Aβ peptide. So as happened after the addition of 25-OHC, the fibrillation of Aβ-42 was restricted to form oligomers and protofibrils as compared to less toxic monomers and mature fibrils.

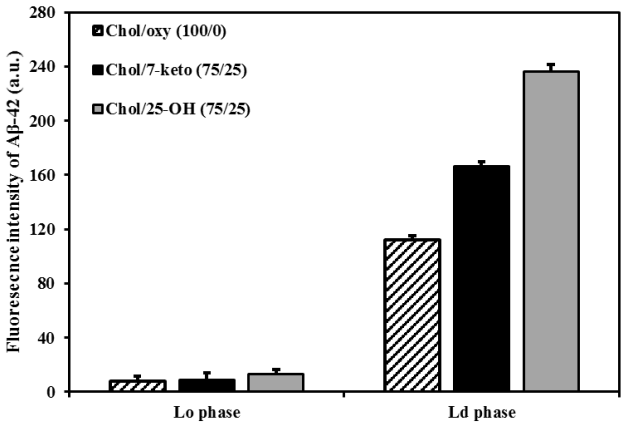

Figure 1. Localization of Aβ-42 in the lateral compartments of membranes in the presence of cholesterol and oxysterols (25-OHC and 7-KC).

Chapter 3, studies on biological membranes, using Jurkat T cells (a leukemic cell line) were performed to assess the effect of oxysterols on the interaction of the peptide with cell membranes. With previous understanding about the oxysterols which suggested induction of peptide insertion into the membrane, increase in excess surface area, enhance surface interaction of Aβ with the membranes. This dissertation have clearly shown that 25-OHC mediates the endocytic transport of protofibrillar Aβ-42 in the cell in correspondence to negative curvature induction by cholera toxin B subunit and monosialoganglioside GM1 interaction. After internalizing into the cells, peptide was transported to ER via microtubules as depicted in the figure 2. As a result, intracellular calcium ions release was observed which induced after disruption of calcium homeostasis by the action of Aβ-42 and oxysterols in a concentration dependent manner. Although Aβ-42 and oxysterols are cytotoxic in nature but they could not cause severe damage to the Jurkat cells under used conditions.

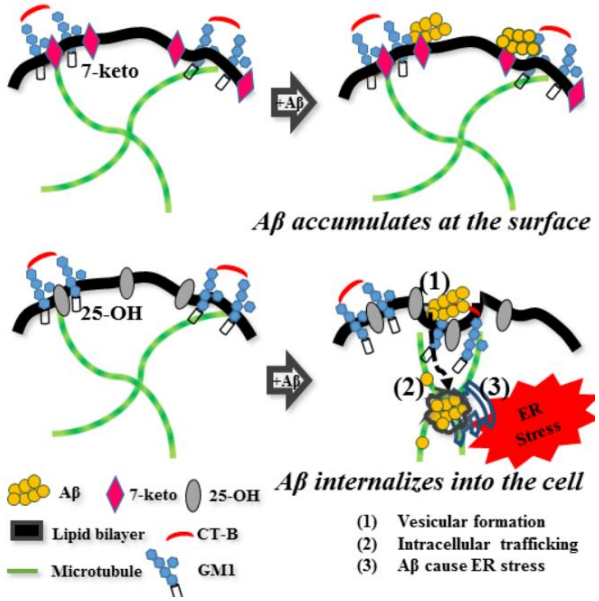

Figure 2. Illustration of the intracellular transport of Aβ-42 in the 25-hydroxycholesterol and cholera toxin B subunit added cells and not in 7-ketocholesterol.

In chapter 4, undifferentiated human neuroblastoma SH-SY5Y cell line which is an analogue of neuronal cells was used to investigate the Aβ-induced toxicity in the progression of Alzheimer's disease. To assess the effect of concentration of peptide, I used different concentrations of protofibrillar Aβ-42 and observed that toxicity of the peptide was dependent upon the concentration of Aβ-42 protofibrils. This was in accordance with a previous study which proposed that the aggregation of peptide varies with its density. At higher concentrations, Aβ-42 was lethal to cells whose effect was increased in the presence of

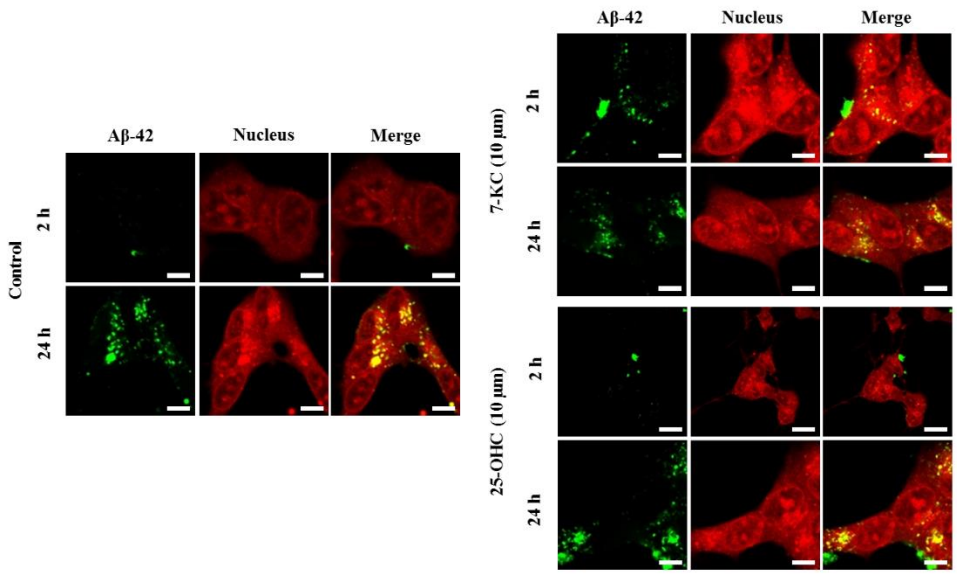

Figure 3. Internalization of Aβ-42 in the human neuroblastoma SH-SY5Y cells in absence and presence of 25-OHC and 7-KC after 2 and 24 h incubation with the peptide. Confocal microscopy was used, Aβ-42 and CT-B are represented by green and red fluorescence, respectively. Scale bars =10 µm.

both oxysterols in time-dependent experiments. Lower concentration of the peptide was used to analyze the internalization affected by oxysterols at different incubation times. Higher amount of protofibrillar Aβ-42 was internalized into the cells in the presence of oxysterols as shown in the figure 3. After internalizing into the cell, protofibrillar Aβ-42 was localized into the ER which indicates the induction of ER stress after accumulation of misfolded proteins in the lumen of ER which was the inspiration and speculation of this study.

In conclusion**,** use of biomimetic and biological membranes provided an advantage to further the understanding about the mechanism behind changes induced by Aβ in AD. Through the findings of this study, a clear view about the risk factors for the disease was represented. Oxysterols, glycosyl chains in the membrane, nano-structures of the peptide were the risk factors studied here. Thus, prevention of oxidation of cholesterol, fibrillation of Aβ-42 and avoiding glycation may be a substantial approach in the treatment of the illness.

Keywords: Amyloid beta, oxysterols, glycation, internalization, membranes.

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List of Publications:

1. **Neha Sharma**, KeangOk Baek, Huong T.T. Phan, Naofumi Shimokawa, Masahiro Takagi. "Glycosyl chains and 25-hydroxycholesterol contribute to the intracellular transport of amyloid beta (Aβ-42) in Jurkat T cells" (FEBS Open Bio, *doi*-10.1002/2211-5463.12234).

2. **Neha Sharma**, Huong T.T. Phan, KeangOk Baek, Naofumi Shimokawa, Masahiro Takagi. "Role of oxysterols in membrane mediated aggregation kinetics of amyloid beta (Aβ-42)" (In preparation).

3. **Neha Sharma**, KeangOk Baek, Naofumi Shimokawa, Masahiro Takagi. "Influence of oxysterols in the internalization of Alzheimer's amyloid beta (Aβ-42) in SH-SY5Y cells" (In preparation).

4. **Neha Sharma**, Yuzuru Takamura, Mun'delanji C. Vestergaard. "Electroanalysis of structure-dependent antioxidant activities of polyphenols" (Minor research, in preparation).

Awards:

Faculty member's choice award for best poster presentation at Jaist poster challenge 2014.

International presentations:

1. "Physics of Cells: From Molecule to Systems (PhysCell 2015)", September 2015, Bad Staffelstein, Germany *(Poster presentation).*
2. "17th SPVM National Physics Conference, 2015 International Conference on Applied Materials and Optical Systems and 2015 International Meeting for Optical Manipulation in Complex Systems" , October 2015, Cavite state university, Cavite, Philippines ***(Invited** oral presentation).*
3. Alzheimer's Association International Conference (AAIC 2016), July 2016, Toronto, Canada (*Poster presentation).*