Doctoral Dissertation Evaluation Review

Degree requested Doctor of Life Science　　Applicant's name　Koolath Sajeer

Examiner:

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Title of Doctoral Dissertation

Synthesis of Chiral Sphingolipids and their Stereochemical Effects on Induction of Neurite Outgrowth and Sphingomyelin Synthase Activity
（キラルスフィンゴ脂質の合成とその立体化学による神経突起伸長及びスフィンゴミエリン合成酵素活性への影響について）

Results of Evaluation of the Doctoral Dissertation (Report)

Sphingolipids are essential components of cell membrane having significant structural and functional roles in the cells. They are first discovered from the brain extract in the 1870s and named after the mythological Sphinx because of their enigmatic nature. Sphingolipids are known to protect cell surface by forming plasma membrane lipid bilayer. They contain a backbone of sphingoid bases, the derivative of amino alcohol sphingosine, sphinganine or phytosphingosine. Sphingolipids help to define the structural properties of membranes and lipoproteins and also have important roles in cell signaling, cell-cell interaction, cell recognition, and help to regulate cell growth and differentiation. Sphingolipids are important biomolecules and they are chiral. Chirality of sphingolipids is very important because they play a very crucial role in the biological system. Most of the drugs are chiral. Naturally, the stereochemistry of most common sphingolipid ceramide has D-*erythro* stereochemistry. So, the chirality of sphingolipids plays a very crucial role in the drug discovery because one enantiomer of a drug may be useful medicine for a disease, but another isomer may be inactive or may be toxic to that disease.

Sphingolipids, ceramide and ganglioside GM3 have attracted intense research interest in drug discovery due to their role in metabolic disorders. A recent study revealed that sphingolipid metabolizing enzymes such as sphingomyelin synthase (SMS) and GM3 synthase are potential drug target to cure metabolic disorders. The deficiency of SMS has been reported to control the development of obesity, fatty liver and type 2 diabetes since then researchers are trying to establish selective and potent SMS inhibitors for the therapeutic application of metabolic disorders. On the other hand, gangliosides play an important role in the induction of neurite outgrowth. This phenomenon is very important for the recovery of the nervous system after injury.

The initial goal of the study was to discover the stereochemical effects of chiral ganglioside GM3 on the induction of neurite outgrowth. To synthesize the stereoisomers of GM3, initially, succeeded in synthesizing and purifying all stereoisomers of sphingosine. Further by using these isomers and sialyl lactose, succeeded in the

synthesis of four stereoisomers of GM3 and performed the cell viability assay. Two different concentrations (10 μM and 40μM) of GM3 isomers are used to perform the neurite outgrowth assay with or without NGF in PC12 cells. Neurite outgrowth activity was measured by staining with Coomassie brilliant blue (CBB) and fluorescence imaging with an inverted microscope and fluorescence microscopy. All four isomers are enhancing the neurite outgrowth in the presence of NGF and without NGF. Surprisingly, L-*erythro* GM3 inducing more neurite outgrowth as compared to the other three isomers.

Further study was continued to understand the effect of sphingolipid chirality on sphingomyelin synthase activity. Successfully synthesized 128 unique stereoisomers of ceramides by efficient solid-phase synthesis. An additional experiment was performed to discover the stereochemical effects of these chiral ceramides on sphingomyelin synthase activity. The cell-based assay of sphingomyelin synthase inhibition in the presence of chiral ceramide suggested that these ceramides have good inhibitory activities (IC_{50} = 0.2 ~ 1 μM) for sphingomyelin synthases 1 and 2. Furthermore, according to heatmap analysis of IC_{50} values, confirmed that most of the unnatural (L)-*threo* stereoisomer ceramide derivatives showed strong inhibitory activities towards SMS1 and SMS2 respectively compared to the inhibitory activities of other stereoisomers.

In conclusion, the author has new findings about stereochemical effects of chiral ceramides on sphingomyelin synthase activity and also synthesis of unnatural GM3 isomers, and their chirality on induction of neurite outgrowth, and these will contribute to the development of novel therapeutic target for metabolic disorders and also nervous system disorder such as Alzheimer's disease, Parkinson's disease etc. Therefore, we acknowledge that the author is qualified to be granted a Doctorate of Life Science from Hokkaido University.