Summary of Doctoral Dissertation

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

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)

Sphingolipids are class of lipids having a backbone of sphingoid base with amide linked fatty acids and having different polar head groups. They are first discovered from the brain extract in the 1870s. Sphingolipids are named after the mythological Sphinx because of their enigmatic nature. They are known to protect cell surface by forming plasma membrane lipid bilayer. These sphingolipids play an important role in signal transmission and cell recognition, involved in many cellular processes such as apoptosis, senescence, differentiation, autophagy etc. Sphingolipids are important biomolecules and they are chiral. Chirality of sphingolipids and understanding their stereochemical effects 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 key role in the drug discovery because one enantiomer of the drug may be useful medicine for a disease, but another isomer may be inactive or may be toxic to that disease.

To understand the stereochemical effects of sphingolipids, synthesis of chiral sphingolipids was performed. Initially, the synthesis of different isomers of GM3 was performed from possible stereoisomers of sphingosine and study their stereochemical effects on induction of neurite outgrowth 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. The result of the neurite outgrowth assay suggests that L-*erythro* GM3 inducing more neurite outgrowth as compared to the other three isomers.

Further confirmation of the importance of stereochemistry of sphingolipids was done by creating the chiral ceramide library. To create the ceramide library, four different isomers of sphingosine was used and derivatize with different types of carboxylic acids by using solid-phase synthesis. Total of 128 chiral ceramides was synthesized and performed the sphingomyelin synthase (SMS) assay to understand the inhibitory activity towards SMS1 and SMS2. The cell-based assay of sphingomyelin synthase in the presence of unique chiral ceramides suggested that L-*threo* type ceramides showing higher inhibitory activities towards SMS1 and SMS2. The libraries of this sort will be a rich source of biologically active synthetic molecules.

In conclusion, synthesis of the four stereoisomers of chiral GM3 and chiral ceramide library was completed. Stereochemical study of four GM3 isomers described that they are inducing the neurite outgrowth and L-*eryhtro* induces more as compared to the other three isomers. On the other hand, stereochemical effects of chiral ceramide on sphingomyelin synthase activity suggested that these ceramides show excellent inhibitory activity on SMS1 and SMS2, especially the unnatural L-*threo* ceramide derivatives showed strong inhibitory activities towards SMS1 and SMS2 respectively compared to the inhibitory activities of other stereoisomers. In the future, these chiral GM3 and chiral ceramides are better therapeutic targets for metabolic disorder and nervous system injuries.