INDEX

CHAPTER I: INTRODUCTION (01-06)

CHAPTER II: REVIEW OF LITERATURE (07-35)

2.1 Introduction of Alzheimer Disease (AD) 07

2.2 Hypothesis of AD pathogenesis 08

2.2.1 Cholinergic hypothesis 08

2.2.2 Tau hypothesis 09

2.2.3 Amyloid cascade hypothesis 12

2.2.3.1 Genetic studies provide evidence for amyloid cascade hypothesis 14

2.2.3.1.1 Amyloid precursor protein (APP) 14

2.2.3.1.2. Presenilins (PSEN) 16

2.2.3.1.3. Apolipoprotein E (ApoE) 16

2.3 Amyloid based therapeutic approaches 18

2.3.1 Decreasing Aβ production 19

2.3.1.1 Inhibition of β secretase 19

2.3.1.2 Inhibition of γ secretase 20

2.3.1.3 Activation of α-secretase 21

2.3.2 Inhibitors of Aβ oligomerization or fibrillization 22

2.3.3. Aβ degradation and clearance 23

2.3.3.1 Neprilysin (NEP) 24

2.3.3.2 Insulin Degrading Enzyme (IDE) 25

2.3.3.3 Angiotensin Converting Enzyme (ACE) 26

2.3.3.4 Cathepsin B 26

2.3.3.5 Endothelin Converting Enzymes (ECE) 27

2.4 ECE isoforms and subcellular localization 29

2.4.1 Endothelin Converting Enzyme-1 (ECE-1) 29

2.4.1.1 Crystal structure study of ECE-1 30

2.4.2 Endothelin Converting Enzyme-2 (ECE-2) 31

2.5 ECE in other disease 32

2.6 Scope and objective of thesis 34

CHAPTER III: MATERIALS AND METHODS (37-52)
3.1 Introduction

3.2 Protein databases
  3.2.1 Protein sequence databases
    3.2.1.1 UniProt (Universal Protein Resource)
    3.2.1.2 GenPept (GenBank Gene Products Data Bank)
    3.2.1.3 Entrez Protein
  3.2.2 Protein Data Bank (PDB)

3.3 Homology modeling
  3.3.1 Template recognition and initial alignment
  3.3.2 Alignment correction
  3.3.3 Model building
  3.3.4 Loop modeling
  3.3.5 Side-Chain modeling
  3.3.6 Model refinement
  3.2.7 Model validation

3.4 Molecular docking
  3.4.1 Docking algorithm
  3.4.2 Scoring Function
  3.4.3 Virtual Screening

3.5 Molecular Dynamics (MD) simulation
  3.5.1 MD simulation algorithms
  3.5.2 Topology
  3.5.3 Force field
  3.5.4 Periodic Boundary Condition
  3.5.5 Thermodynamic ensembles
  3.5.6 Energy minimization
  3.5.7 Water model
  3.5.8 Lipid model
  3.5.9 Molecular dynamics simulation setup
    3.5.9.1 A starting structure
    3.5.9.2 System preparation
    3.5.9.3 Thermodynamic ensembles
3.5.9.4 Position restrained MD
3.5.9.5 Fully unrestrained MD

CHAPTER IV: RESULTS AND DISCUSSIONS (53-130)

4.1 Homology modeling and MD simulation studies of hECE-1 with phosphoramidon

4.1.1 Introduction

4.1.2 Materials and Methods

4.1.2.1 Homology Modeling
4.1.2.2 Molecular Dynamics Simulation of whole hECE-1 and phosphoramidon
4.1.2.3 Hydrophobicity and Molecular Electrostatic Potentials (MEPs) calculations

4.1.3 Results and Discussion

4.1.3.1 Analysis of homology model of 10 missing residues of whole hECE-1
4.1.3.2 Structure of whole hECE-1 after MD simulation
4.1.3.2.1 S1 Pocket of whole hECE-1
4.1.3.2.2 S2 Pocket of whole hECE-1
4.1.3.2.3 Simulation stability of whole hECE-1 with phosphoramidon
4.1.3.3 Phosphoramidon and whole hECE-1 interactions
4.1.3.3.1 Hydrophobicity and electrostatic calculations
4.1.3.3.2 Interactions of phosphoramidon with whole hECE-1 and zinc
4.1.3.3.3 Interactions with metal ion during MD simulation

4.1.4 Conclusions

4.2 Studies on other protease inhibitors to probe active site of hECE-1 using virtual screening and MD simulation

4.2.1 Introduction

4.2.2 Material and Methods

4.2.2.1 Model preparation and virtual screening
4.2.2.2 Molecular docking using AutoDock
4.2.2.3 Molecular dynamics simulation

4.2.3 Result and discussion

4.2.3.1 Model preparation and virtual screening
4.2.3.2 Docking using PyRx and AutoDock
4.2.3.3 Molecular dynamics of whole hECE-1 with inhibitor
4.2.3.3.1 Structure of whole hECE-1 after simulation
4.2.3.3.2 Ligand interaction with whole hECE-1
4.2.3.3.3 Interactions with metal ion during MD simulation

4.2.4 Conclusion

4.3 Molecular docking study of hECE-1 with wild type and mutant Aβ peptides
4.3.1 Introduction
4.3.2 Materials and Methods
4.3.2.1 Molecular docking of whole hECE-1 and amyloid beta peptide
4.3.3 Results and Discussion
4.3.3.1 Molecular docking of Amyloid Beta (Aβ) peptide and whole hECE-1

4.3.4 Conclusions

4.4 Insights into subsite recognition and cleavage mechanism of Aβ peptide
4.4.1. Introduction
4.4.2. Materials and methods
4.4.2.1 Receptor and ligand preparation
4.4.2.3 Molecular docking of hECE-1 and amyloid beta peptide
4.4.2.4 Molecular dynamics simulation of hECE-1 and amyloid beta peptide
4.4.3 Results and Discussion
4.4.3.1 Molecular docking of Amyloid Beta (Aβ) peptide and hECE-1
4.4.3.2 MD simulation of Aβ with hECE-1
4.4.3.2.1 hECE-1 and Aβ peptide interaction
4.4.3.2.2. The zinc ion coordination during molecular dynamics
4.4.3.3. Proposed cleavage mechanism of Aβ peptide

4.4.4 Conclusion

4.5 Structural analysis of membrane bound hECE-1 dimer with Aβ1-42 peptide using molecular modeling techniques
4.5.1 Introduction
4.5.2 Material and Methods
4.5.2.1 Homology modeling of hECE-1
4.5.2.2 Membrane bound hECE-1 dimer preparation
4.5.2.3 Molecular dynamics simulation of hECE-1 dimer embedded in lipid bilayer
4.5.2.4 Molecular docking of hECE-1 dimer and Aβ1-42 peptide

4.5.2.5 Binding free energy calculations of hECE-1 and Aβ1-42 peptide complex

4.5.3 Result and discussion

4.5.3.1 Homology modeling of hECE-1

4.5.3.2 Homodimer of hECE-1 embedded in lipid bilayer

4.5.3.3 MD simulation stability and Secondary structure calculation of hECE-1 dimer

4.5.3.4 Overall structure of hECE-1 dimer after MD simulation

4.5.3.5 Normal mode analysis of substrate entry/exit site

4.5.3.6 Molecular docking and MD simulation of hECE-1 and Aβ1-42 complex

4.5.3.7 Analysis binding free energy of hECE-1 and Aβ1-42 complex

4.5.4 Conclusion

CHAPTER V: SUMMARY AND CONCLUSIONS

5.1 Summary

5.2 Conclusion

CHAPTER VI: REFERENCES

CHAPTER VII: RESEARCH PUBLICATIONS