An In-Silico Study of Inhibition of Alpha-Synuclein Aggregation by Glycol Stearate for Treatment of Parkinson's And Gaucher's Disease

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***Abstract*— Aggregation of alpha-synuclein is an eminent cause of neurotoxicity in both Parkinson's and Gaucher's disease. Also, it is evident that mutated non-functional glucocerebrosidase in Gaucher's diseaseencourage alpha-synuclein accumulation,as its standard substrate glucocerebroside stabilizes the aggregated complex, thereby increasing the risk of Parkinson's. The peptide region which actively participates in aggregation ranges from 64-100 residues of alpha-synuclein. Glycol stearate, a commonly used emulsifier in food and cosmetics, based on its ADMET properties shows high blood brain barrier permeability and LD_{50} of 5000mg/Kg. In the present study, molecular docking was performed to analyze the structural inhibition of alpha-synuclein aggregation by glycol stearate. Our results proved that glycol stearate binds to alpha-synuclein inside the active site with a binding energy of -5.3kcal/mol. Hence, inhibition ofalpha synuclein aggregationby a potent inhibitor like glycol stearate can be an effective approach for treating major pathologies of both dreaded diseases.**

***Keywords*— alpha-Synuclein, Gaucher's Disease, Parkinson's Disease, Glycol stearate, Molecular Docking**

I. INTRODUCTION

Neurodegenerative diseases are endangering a remarkably large population around the world. They result from neuronal cell death in various parts of brain and spinal cord due to innumerable associated pathologies [1]. Out of manyneurodegenerative diseases Parkinson's disease being the second most dreaded one, is affecting about 7 to 10 million people around the globe [2]. Major symptoms of the disease comprise tremor, bradykinesia, postural instability and rigidity which results from significant reduction of dopaminergic neurons in substantia nigra and associated regions due to the accumulation of alpha synuclein inclusions (Lewy bodies) [3, 4]. Along with these regions such accumulations can be found in other regions of central nervous system thereby causing behavioral losses and dementia [4, 5]. Another characteristic pathology associated with Parkinson's is accumulation of tau protein into neurofibrillary tangles which hamper the microtubular stabilization and axonal transport in neurons and hence causes cell death. The formation of tangles is due the hyperphosphorylation of different isoforms of tau protein containing varying number of microtubule binding motifs. Past research had made it evident that high toxic levels of alpha synuclein accumulation leads to hyperphosphorylation of tau protein and thus enhance the adverseness of the disease [6, 7].

Though genetic mutations invarious genes like *alpha synuclein, DJ-1, PTEN induced putative kinase 1, parkin and Leucine rich repeat kinase 2* are reported to be the leading cause for the disease, there are variety of other genetic mutations responsible for the same [8]. Apart from the genetic changes there are evidences of inter-relation between pathologies of neurodegenerative diseases in which the pathology of one disease can enhance the risk of the other disease. One such proved example is of Gaucher's disease (GD) which is evident to increase Parkinson's risk in patients.GD is a recessive autosomal disorder which results into lysosomal glucocerebrosidase enzyme deficiency and hence cause accumulation of enzyme's standard substrate glucocerebroside [9]. Manning-Bog et al., in 2009 provided evidence for the above-mentioned inter-relationship of GD and Parkinson's for the first time in their study of inhibition of glucocerebrosidase by conduritol B epoxide in neoblastoma cells and mouse model. Their findings proved that inhibition of the enzyme enhanced the levels of alpha synuclein in both cell culture and mouse model. In animal model they concluded transformation of normal distribution of alpha synuclein to accumulated form by using western blot and confocal microscopy on sections of coronal brain of substantia nigra [10]. Mazzulli et al., in their deep study revealed many aspects associated with enhanced alpha synuclein accumulation in GD patients. They reported reduction in lysosomal degradation in glucocerebrosidase knocked out primary cultures, which improved the alpha synuclein accumulation up to 1.8 times hence, enhancing aggregational neurotoxicity. Also, glucocerebroside, the substrate of glucocerebrosidase, pose great impact on the formation of alpha synuclein amyloid by stabilizing high molecular weight soluble oligomeric intermediates which at last form insoluble forms of aggregates. In the same study they also reported that alpha synuclein inhibits the activity of glucocerebrosidase in lysosomes in idiopathic form of Parkinson's [11]. Therefore, there exist a strong relation between the two dreaded neurodegenerative disorders and focusing on the common pathology of alpha synuclein accumulation can provide potential treatment for both the disease under concern. Structural inhibition of alpha synuclein accumulation can be a highly potent approach for the same. Past studies have proved

that hydrophobic region of alpha synuclein sequence ranging from 61 to 100 plays important role in polymerization of the protein. Giasson et al., also reported mid region of alpha synuclein to be highly amyloidogenic and possess significant efficiency to form fibrils readily.Thus, finding potential inhibitors which can bind in this region can be of great significance in this concern.

Molecular docking studies have made great contribution in finding potent treatments for majority of disease by analyzing drug target interactions and associated structural inhibition profiles [12]. Such in-silico analysis provides a significant scope infinding potential drug leads. A variety of web and system basedtools like Autodock, Autodock Vina, Swiss dock, Lead IT, HEX, GOLD, GLIDE etc. are available which can perform interaction analysis by molecular docking [13]. For a compound to be a potent drug along with structural inhibition potential, it should possess favorable ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties. In case of central nervous system drug prediction, it becomes highly likely to find a lead which has potential to cross the blood brain barrier in intact brain. Lipinski's rule of 5 act as standard for considering a lead, good as CNS drugbut still there are many candidates in market which are highly effective CNS drugs but violate one of the parameters of rule of 5 [14]. Glycol stearate is an extensively used emulsifying agent in food and cosmetic industries and is known to be very less toxic. It is widely used in personal care products like face creams, shampoos etc. In food industry, it is a frequently used food additive which act as emulsifier and enhance smoothness in texture, prevents phase separation in food formulations and enhance shelf life of food. Hence, its emulsifying property can also be of great importance in dispersing the aggregates of alpha synuclein in different parts of brain [15, 16, 17]. So, in present study we hypothesize that glycol stearate can be a potential lead for inhibition of alpha synuclein aggregation and accumulation. Hence, to prove our hypothesis we performed analysis for ADMET properties to verify its potential to act as drug and then molecular docking was performed to deeply investigate its structural inhibition potential against the accumulation of leading target alpha synuclein for the treatment of the two highly complex neurodegenerative disorders.

II. MATERIALS AND METHODS

a. ADMET, bioactivity and molecular properties analysis

ADME properties of glycol stearate were analyzed by using online server Swiss ADME developed by Swiss Institute of Bioinformatics [18]. PROTOX II a web-based tool by Charite University of Medicine Germany was used for toxicity analysis of the compound [19]. To get the bioavailability scores MolinspirationCheminformatics server was used [20].

b. Molecular Docking

Glycol stearate chemical structure was made by employing a system based Chem Sketch software of ACD labs (figure 1)[21]. Molecular docking analysis was performed by using Autodock Vina of MGL tools 1.5.6 [22]. The PDB structure of the compound was made by using online smile translator. The PDB structure of human alpha synuclein protein (PDB 1XQ8) was procured from Protein Data Bank RCSB (Research Collaboratory for Structural Bioinformatics). Water molecules and Hetroatoms were removed from the PDB file of protein and all non-polar hydrogen atoms were merged. Lamarkianalgorithim based on local adaptive search was used as search parameter. Electrostatic interaction, Van der walls forces, hydrogen bonding and entropy losses were employed to estimate energy-based scoring function. Kollman charges of -16 were added to the protein structure. The parameters of Genetic Lamarkian algorithm used for analysis were: number of runs=30, population size=150, maximum number of evaluations=2500000, number of generations =27000,crossing over rate = 0.8 and rate of mutation in gene=0.02. Docking was performed by placing the grid on the active site region responsible for alpha synuclein aggregation comprising residue range of 61 to 100. The grid size of 98, 126, 50 along X, Y, Z axis respectively with 0.75 as spacing was used. The grid centers were allotted as X = 234.671, Y = 24.769, and Z = -20.363 and RMS cluster tolerance used was 2 Å.

III. RESULT AND DISCUSSION

Aggregation of alpha synuclein contributes to pathologies of both Parkinson's as well as Gaucher's disease and leads to the adversity of diseases by promoting cell death in parts of brain [11]. Prevention of aggregation can provide great scope for treating the diseases to a large extent by reducing major symptoms of diseases. Structural inhibition by lead compound against aggregation can prevent or dislodge the accumulated arrangements of synuclein and can work for the purpose. Glycol stearate is commonly used emulsifying agent in many cosmetics and some food products and thus is safe to be used in vivo. Our results of physiochemical properties, ADME properties and bioavailability score for the compound proved its potential to be a drug like candidate for central nervous system drug (Table 1). Its ultimate capability to pass blood brain barrier proves it to be a compound of immense importance in concerns to CNS drugs. The toxicity prediction results proved it a compound of toxicity class 5 with predicted LD_{50} of 5000mg/Kg. Keeping concerns with the above-mentioned results we performed molecular docking to analyze structural inhibition of aggregation of alpha synuclein by glycol stearate. Our docking results showed that glycol stearate binds the alpha synuclein protein in the active region taking part in aggregation with a high negative binding energy of -5.3kcal/mol and thus has great potential to hinder and prevent accumulation of alpha synuclein into stable fibril complexes there by providing high scope for treatment of major pathology in Prakinson's and Gaucher's disease (figure 2A). The major residues involvedwith which the compound interacts range from VAL95 to ASN103.Binding of the lead at the active site will hinder the aggregation of protein into fibrils and will result in reduced accumulation of it. In present analysis we also found potential compounds like lutein and

violacein which interacted with the active site residue with high negative binding energies of -5.6 and -6kcal/mol respectively (figure 2B, 2C). Although both are natural compounds with good antioxidant properties which in turn can be beneficial to combat oxidative stress, they are not capable to cross the blood brain barrier in intact brain and need to be encapsulated into a carrier moiety to pass the same (Table 1). Jayaraj et al., 2013 in their molecular docking study on inhibition of aggregation of alpha synuclein reported stimovul as potent inhibitor with binding energy of -4.5kcal/mol,interacting with the active site, which is less as compared to our result thus proving our compound a better candidate for the treatment [23]. Reddy and Parthasarathy in their molecular docking study of alpha synuclein reported delonal as a potent interacting drug with a CDOCKER energy of -45.61 but the interacting region of protein was not reported [24].

Hence, present study put forward highly potential CNS drug compound glycol stearate for the inhibition of alpha synuclein aggregation, the major pathology responsible for neurodegeneration, in the two dreaded diseases Parkinson's and Gaucher's diseases. Also, it set together some promising natural compounds (Lutein and Violacein) which can be employed for the same with the development of specific drug delivery system to pass the blood brain barrier. Thus, further research in this concern can lead to a highly effective and efficient treatment to deal with the huge population which is being victimized by the most prevalent neurodegenerative diseases.

IV. CONCLUSION

Parkinson's disease is the second most widespread neurodegenerative disorder with major associated pathology of aggregation of alpha synuclein. Gaucher's disease a lysosomal glucocerebrosidase deficiency disorder is reported to promote such aggregations and lead to risk of Parkinson's. In turn synuclein accumulation inhibits the enzyme in concern and lead to more adverse condition. Present study enumerates the potential of probable compounds, by molecular docking analysis, which can inhibit such aggregations by binding in the significant region of alpha synuclein responsible for it. Our results proved glycol stearate, a highly potent compound with good emulsifying ability, as an inhibitor of aggregation of alpha synuclein in neural cells and a treatment to the diseases in concern. Hence extensive further is demanded to treat the two till date incurable neurodegenerative diseases.

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TABLE 1: PHYSIOCHEMICAL PROPERTIES, ADME PROPERTIES AND BIOAVAILABILITY SCORES OF GLYCOL STEARATE

Physiochemical properties	
Formula	C20H40O3
Molecularweight	328.53g/mol
Num. heavy atoms	23
Num. arom. Heavyatoms	0
Fraction Csp3	0.95
Num. rotatableBonds	19
Num. H-bond Acceptors	3
Num. H-bond Donors	1
Molar Refractivity	100.70
TPSA	46.53 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	4.40
Log $P_{o/w}$ (XLOGP3)	7.91
Log $P_{o/w}$ (WLOGP)	5.78
Log $P_{o/w}$ (MLOGP)	4.25
Log $P_{o/w}$ (SILICOS-IT)	6.59
Consensus Log $P_{o/w}$	5.79
Water solubility	
Log S (ESOL)	-5.61
Solubility	8.14e-04mg/ml ; 2.48e-06 mol/l
Class	Moderately soluble
Log S (Ali)	-8.74
Solubility	6.03e-07 mg/ml ; 1.83e-09 mol/l
Class	Poorly soluble
Log S (SILICOS-IT)	-6.64
Solubility	7.49e-05 mg/ml ; 2.28e-07 mol/l
Class	Poorly soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-2.69 cm/s
Druglikeness	
Lipinski	Yes; 1 violation:MLOGP>4.15
Ghose	No; 1 violation: WLOGP>5.6
Veber	No; 1 violation: Rotors>10
Egan	Yes
Muegge	No; 2 violations: XLOGP3>5, Rotors>15
Bioavailability Score	0.55
Medicinal chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 2 violations: Rotors>7, XLOGP3>3.5
Synthetic accessibility	3.34
Bioavailability score	
GPCR ligand	0.04
Ion channel modulator	-0.04
Kinase inhibitor	-0.08
Nuclear receptor ligand	0.12
Protease inhibitor	0.09
Enzyme inhibitor	0.13