Targeting α-synuclein synthesis, aggregation, and propagation as a potential therapy for Parkinson's disease

Swati Sharma[1], R Vadivelan[1*], Richu Babu[1], T Jyothsna[1], Arun T Nair[1]

[1]Department of Pharmacology, JSS College of Pharmacy, Ooty, Jagadguru Sri Shivarathreeshwara University, Mysuru, India

Abstract:

Based on the latest advancements in Parkinson's disease (PD), α-Synuclein has been the most recent and an influencing target in its study. Accumulation of misfolded Alpha-synuclein (α-syn) has been the main culprit and the hallmark of the disease. In past two decades, scientists have found that α-synuclein has the tendency of self-propagation leading to the extension of PD. α-Syn was considered a major player in the disease when it was noted to be the main component of Lewy bodies. Lewy bodies (Lewy bodies) are the Intracellular inclusions found in the central nervous system comprised of α-Syn protein as one of the main constituents. Many *in vitro* and *in vivo* studies show that α-syn is able to undergo a conformational change which can spread from one cell to another and lead to the formation of prion-like properties. Animals used for the study of aggregation of the protein must be able to show the loss of dopaminergic neurons, decreased dopamine content and must show changes in the movement. In this article, we discuss the normal functions of α-Syn and its role in the progression of the disease. We also discuss animal models used for α-Syn induction in PD studies.

KEYWORDS: α-Synuclein, Parkinson's disease, Dopaminergic neurons, Lewy bodies, Substantia nigra pars compacta.

INTRODUCTION:

In the pathogenic study of Parkinson's disease (PD), the role of α-synuclein started gaining attention since 1997 when it was found that a mutation in SNCA gene i.e. responsible for the encoding of α-synuclein, was one of the leading causes of PD. Parkinson's disease is the second most common neurodegenerative disease after Alzheimer's disease characterized by the loss of dopaminergic neurons in substantia nigra pars compacta (Snpc) and by the presence of Lewy bodies. α -synuclein (α-syn) is one of the major components of Lewy bodies and is linked neuropathologically and genetically to PD.[1] α –Syn (also known as nonamyloid β- component protein or NACP) is a neuronal protein found in the presynaptic terminus. Since this protein was known to be present in both synapses and nuclei, the protein was termed as synuclein but the following studies denied the presence of α-synuclein in the nuclei.[4,10] α-synucleinuclein is abundant in human brain. The highest level is found in the hippocampus, Substantia nigra, neocortex, cerebellum, and thalamus. Small amounts of this protein can be found in muscles, heart, and tissues.

Due to various In vitro experiments, In vivo experiments and studies for genetic mutation, a link between synucleinopathies and α-synuclein has been created. α-synuclein has been the main focus in understanding various neurodegenerative disorders called synucleinopathies including PD. An important feature of synucleinopathies is that they consist of proteinaceous intracellular bodies containing α-syn in aggregated form. These bodies exist in different forms in different synucleinopathies. There are various studies that confirm that the major cause that leads to synucleinopathies is the formation of aggregates due to misfolding of the protein. The formation of an aggregate involves nucleation as the initial step, an intermediate oligomeric state, and a final fibrillar amyloid formation. α-synucleinuclein in aggregated form has been found in the patients with synucleinopathies which is a characteristic of neurodegeneration and can lead secondary processes like neuroinflammation and cell death. These findings led to many further types of research which were able to identify α –syn in many other neurodegenerative disorders.[2,3]

Firstly, α-syn was identified in electric ray (*Torpedo californic)* by Maroteaux and his co-workers in 1988. The α-synuclein cDNA of the rat was cloned in the same year whereas; the mouse cDNA had not been cloned before 1998.[5] The relationship between α-synuclein and PD can be studied using appropriate animal models. An ideal PD model should exhibit the signs of idiopathic PD i.e. bradykinesia, resting tremor, postural impairment, akinesia, muscle rigidity, a relatively selective loss of Dopaminergic neurons and the presence of Lewy bodies and Lewy neurites. Other features include a reduced mitochondrial complex I activity and high concentration of ROS.[6] A perfect animal model can be created using neurotoxins that are able to inhibit mitochondrial complex I i.e. MPTP, rotenone or by the overexpression of α-synuclein. Both the methods lead to accumulation of α-synuclein the neurons. In this article, we focus on these models and the latest advancements that have been made over the years focusing on α-synuclein as the main target.

STRUCTURE OF α-SYNUCLEIN

A lot of studies have been carried out on the structure of α-Syn. The primary structure of the protein consists of three regions: (1) an amino (N) terminus (1–60 residues) consisting of apolipoprotein lipid-binding motifs and seven imperfect amino acid repeats (KTKEGV), important in the formation of α-helix (2) a hydrophobic region present in the centre (61–95 residues), also known as NAC (Non- Ab component), important in aggregation of protein and (3) A carboxyl (C) terminus which is negatively charged and proline-rich.[2,7](Figure 1)

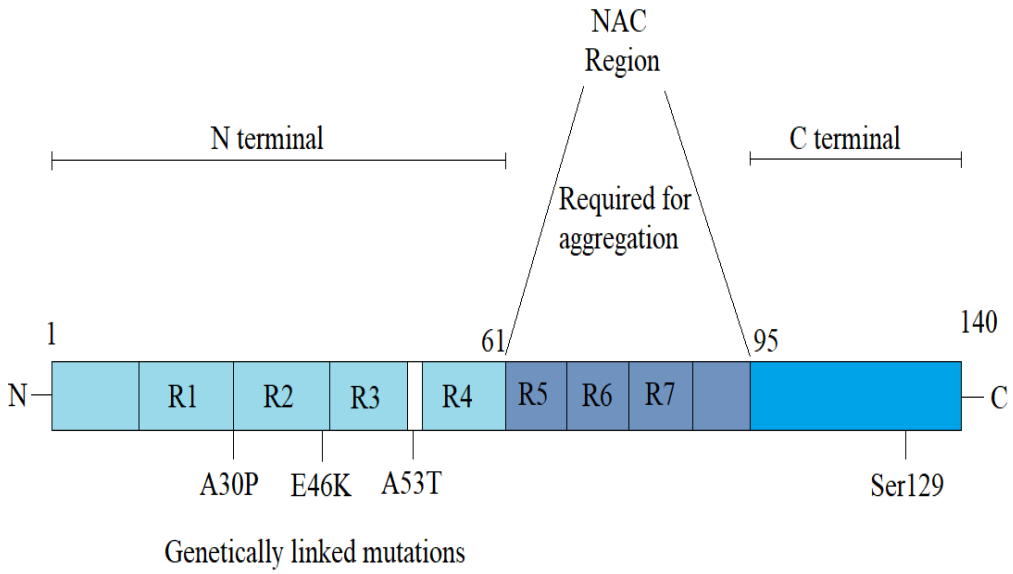

Figure 1: Structure of α-synuclein showing the NAC domain

The secondary structure depends upon the environment, α-synuclein is made up of 140-amino-acids having random coiling and a few α-helixes and β-sheets. Random coiling structure gives a broad peak by FTIR spectroscopy at 1650 cm-1. Circular Dichroism spectra show 70% random coiling and 2% alpha-helical content. This protein has a highly unstructured conformation. The molecular mass of α-synuclein is not consistent due to an extended structure. The molecular weight found by SDS-PAGE is 19 kDa, having a high amount of negatively charged residues at the C-terminal. [3]

Pathogenically, 3 α-synucleinuclein point mutations are known i.e. A30P, E46K, and A53T. All the three mutations have a capability of forming fibrils more quickly than wild-type proteins. Dopamine transporter-mediated alterations are caused by neurotransmission when human A53T α -Syn expressed in mice. Reduction in lipid binding can be

caused by A30P α -Syn. Introduction of mutant E46K to a cell leads to the formation of α -Syn aggregates in an amount more than the ones caused by mutants A30P and A53T. [12]

α-SYNUCLEIN AGGREGATION AND THE THEORY OF PROPAGATION

Aggregation of α-synuclein occurs naturally. The monomeric α -Syn bound to the membrane adopts a α-helical conformation which changes to membrane-bound β-sheets at higher concentrations. This membrane-bound β-sheets form oligomers including amyloid pores by self-association. The intermediate species formed during the process of the formation of fibrils and their aggregates, are highly toxic and can affect the mitochondrial function and cause degradation of the protein leading to neurodegeneration. The process of aggregation is shown in Figure 2. α-synuclein oligomers, monomers, and the fibrils can be transferred from cell to cell and can spread the disease to different regions of the brain. Other mechanisms by which the disease can spread to various brain regions include penetration, endocytosis, by membrane receptors and by Trans-synaptic transmission. [13]

Various environmental and biological factors like low pH, lipid membranes, metals, Fe_3O_4 particles and SDS are responsible for the induction of aggregation. Nitration can also lead to the formation of stable aggregates. Heavy metals like lead, copper, mercury, iron, zinc have been found to enhance the aggregation of α-synuclein. Substansia nigra consists of iron in large concentration. In vitro, ferrous iron catalyzes the formation of α-synuclein aggregates in the presence of H_2O_2 by Fenton reaction.[3] Dopamine also modifies the alpha-synuclein aggregation. The dopaminergic neurons attribute towards the enhanced presence of soluble oligomers, DOPAC also inhibits the formation of fibrils by binding and stabilizing the protofibrils. [3,7].

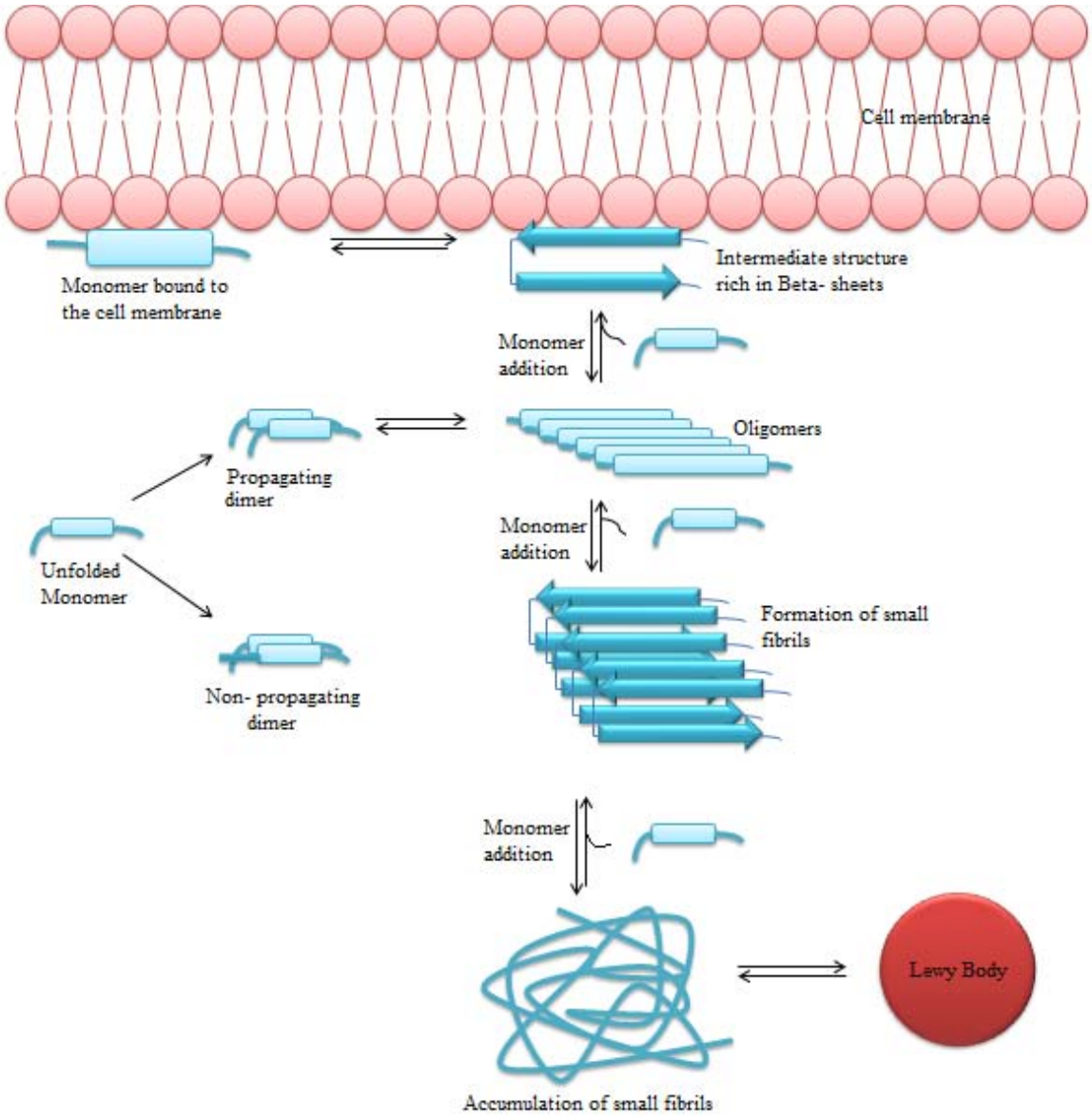

Figure 2: Process of aggregation and formation of Lewy bodies

In his work, Braak and his co-workers explained the propagation of α-synuclein. Animal models for PD were observed with the transmission of α-synuclein from cell to cell. [9] Also, α-synuclein positive Lewy bodies were developed in grafted neurons, observed in 2 human subjects. The dopaminergic neurons grafted developed pathology of Lewy bodies after 11- 14 years of implanting in the PD patients [15]. This led to the 'prion-like hypotheses.' According to this hypothesis, α-synuclein is transferred to the extracellular space and then to the nearby cell from the host cell. The process of protein misfolding starts again in the new cells and new Lewy bodies are formed. [9]

The exact mechanism of propagation of α-synuclein is still undiscovered. α-synuclein is present extracellularly in humans as well as in the cell culture in both monomeric and oligomeric form. The conditions which lead to disturbances and misfolding of the protein are responsible for causing an increase in α-synuclein, extracellularly. α-synuclein can lead to neurotoxicity inside the cytoplasm of the nearby cells. It can also cause damage to the cells present in the extracellular space. Thus, α-synuclein can propagate inside the brain in many possible ways. An

experiment was performed on transgenic mice using this idea. α-synuclein was overexpressed in the mice and α-synuclein rich accumulation was seen in the neurons. Many other similar studies were performed using this idea. Regardless of the progress, greater attempts are needed to obtain a clearer picture in this aspect. [14]

FUNCTIONS OF α-SYNUCLEIN IN PRESYNAPTIC TERMINAL

Despite all the progression in the study of PD, the normal functions of α-synuclein in presynaptic terminal still remains a secret for the researchers. Due to its unstructured form, it keeps moving in the cytosol because of which the study of this protein has been difficult. The interaction of α-synuclein with the presynaptic neuronal membrane shows that almost all of the α-synuclein functions are related to the synapse.

Neurotransmitter release

Localization of α-synuclein in the synapse and co-localization in the synaptic vesicle pool suggests that α-synuclein plays a major role in the neurotransmitter release.[21]Various studies have been done using knockout animals and even by the overexpression of α-synuclein. Some studies reported the release while some showed the inhibition of the neurotransmitter. Some of the studies showed no effect on the release. [22, 23, 24] In a study, α-synuclein knockout mice showed an increase in the dopamine stores and decreased synaptic vesicles endocytosis. [16] α -syn which is present in the presynaptic terminal in a huge amount interacts with synaptic vesicles docking and trafficking of the vesicles. Overexpression of α-synuclein though results in a decrease in this trafficking causing a decrease in neurotransmitter release.

Protein interaction

In the presynaptic terminal α-synuclein is known to interact with various proteins. The known interactions include Synaptobrevin- 2 (VAMP2), which has a role in synaptic exocytosis, SNARE complex assembly, chaperone proteins, protein kinase C (Protein kinase C), tau protein, dopamine transporter protein (DAT). α-synuclein, by this interaction, have many functions like remodeling of the membrane, modulation of release from synaptic vesicles, the formation of clusters os synaptic vesicles and maintaining its pool etc. [17, 18, and 21]

Molecular chaperone-like activity

α-synuclein is structurally and functionally homologous with the 14-3-3 family of molecular chaperone so it can also be considered as a chaperone protein. [18] α-synuclein can bind to the same targets as this family. The target binding sites can be phosphorylated by these 14-3-3 proteins consisting of Protein kinase C, Bcl-2- associated death promoter, and Extracellular signal-regulated kinas. For α-synuclein to act as chaperone, N and C- terminals are necessary. The domain of N- terminal helps in substrate protein interaction of α-synuclein to form a large complex whereas C- terminal helps in the solubilization of this large complex. [20]

Impact on dopamine biosynthesis

Due to the inhibition of tyrosine hydroxylase by α-synuclein, the synthesis of dopamine can be modulated and controlled. Therefore, the aggregation of α-synuclein can cause an increase in dopamine levels and can lead to oxidative stress. Protein phosphatase 2 A is responsible for inhibition of tyrosine hydroxylase activity. Protein phosphatase 2 A interacts with α-synuclein and Protein phosphatase 2 A phosphatase is activated, causing dephosphorylation of TH-Ser40 residue and therefore inhibiting it. [20]

Reduction in Protein kinase C activity

Protein kinase C affects many mechanisms of apoptosis by phosphorylating target proteins. It triggers a proteolytic cascade of the dopaminergic neurons. α-synuclein has a capability of down-regulating Protein kinase C and protecting the dopaminergic neurons from apoptosis. α-synuclein deactivates NF-kB. This deactivation leads to a reduction in the transcription process of Protein kinase C. [20]

Interaction with calmodulin

In both in vitro and in vivo conditions, α-synuclein has been observed to interact with both mutant and wild-type α-synuclein. Calcium ions can modulate the function α-synuclein by causing an interaction between α-synuclein and calmodulin. This interaction leads to α-synuclein fibrillation. Calmodulin also inhibits GRK5 when α-synuclein is not present. [25]

Maintenance of SNARE structure

The release of neurotransmitters from the synaptic vesicles depends upon the proteins responsible for the membrane fusion. *N*□ethylmaleimide SNARE complex is assembled and disassembled each time the neurotransmitter is released. This process goes along with the intermediate formation of SNARE protein structures. As this process occurs, α-synuclein involves the maintenance of these structures. Therefore, α-synuclein deficiency may lead to reduced SNARE complex assembly which can cause neuronal damage. [20]

Action as an anti-oxidant

Unsaturated phospholipids undergo oxidation in the dopaminergic neurons which damages both intracellular and cellular membranes. This damage is caused due to dopamine metabolism in these cells. Thus, the dopaminergic membranes are abundant with unsaturated FA. α-synuclein, in monomeric form can interact with these lipid membranes and prevent their oxidation but not in the form of fibrils. Thus, α-synuclein can protect the dopaminergic membranes from oxidative stress. [26]

α-SYNUCLEIN IN PARKINSON'S DISEASE

SNCA gene which encodes for α-Syn has 3 point mutations A30P, E46K, and A53T. The expression of PD was observed were gene locus multiplications occurred in the SNCA. [10, 27]

Due to this linking of PD and SNCA, the researchers were able to generate antibodies against the protein and used them in sections of the brains of the patients with PD. Today in the study of PD, the immunohistochemistry of α-synuclein is used as a standard for its neuropathological study.

A lot of studies were performed on α-synuclein and regarding its role in PD. Three main observations were made from these studies: (1) α-synuclein is not only confined in the pathogenesis of PD and can lead to different synucleinopathies such as dementia with Lewy bodies, Alzheimer's disease, multiple system atrophy etc. (2) α-synuclein is not restricted to only one area and is found various regions of the brain in PD patients (3) α-synuclein can be present both in neuritic pathology and in cell body. [1, 10]

Braak et al. in 2003 took a group of similar tissues from brain bank which consisted of both control and PD brains and performed immunohistochemistry with antibodies for α-synuclein. Some of the brains were found with accumulated α-synuclein. The aggregation of α-synuclein was developed in these areas. From this study, Braak and his co-workers came to a conclusion that firstly the olfactory bulb is affected, followed by the dorsal nucleus of the vagus in an ascending order. The substantia nigra is the only part that gets affected in stage 3. Stage 4 to 6 mostly involves cortex regions and limbic system. The hypothesis generated was able to explain a lot of but not all of the conditions that appeared in PD. [1, 10, and 28]

Following this study, two different studies by Li et al. and Kordower et al. in 2008 observed that after some years of grafting the striatum part of PD patient's brain with mesencephalic neurons that are embryonic were seen to develop LB's. This lead to the use of words like ''prion-like'' for explaining the pathogenesis of α-synuclein. Another group in 2008 reported that LB's developed in a patient after 14 years of grafting. Brundin et al. explained this condition by plotting the difference between the development and absence of Lewy bodies in the grafted patients. They said that that the development might be affected by the grafting environment, protocols of histology used or individuals PD patients. [7]

α-SYNUCLEIN TOXICITY

Oligomers that cause α-synuclein aggregation are capable of mediating α-synuclein toxicity. α-synuclein can be converted into toxic form by the interaction between lipid molecules on processes like S129 phosphorylation. In vitro, α-synuclein aggregates consisting of β- sheets can be formed by maintaining a high concentration of protein, increased time of incubation at 37^{o}, the addition of appropriate metal ions, dopamine addition or nitration. Heteropolymers or homopolymers are formed by the cross-linking of α-synuclein. According to a new theory, amyloid form helps in avoiding the toxic oligomer formation. α-synuclein can produce toxicity in its prefibrillar form whereas the protofibrils produced have the capability of disturbing the membrane and produce toxicity by the influx of calcium ions. It has also been suggested that the presence of α-synuclein in high level can also lead to toxicity. α-synuclein loss can also cause neurotoxicity. α-synuclein increases the level of dopamine and produce toxicity by inhibiting tyrosine hydroxylase. Its loss can also increase membrane permeability by increasing PLD_2 activity. [3, 13]

THERAPIES AGAINST α-SYNUCLEIN AGGREGATION

To target α-synuclein during its movement between the cells is a useful therapeutic approach. Another suitable therapeutic approach involves interference with the aggregation and the misfolding. PD study has undergone a lot of development using these steps as its target.[9]

Heat shock proteins

Heat shock proteins are classified according to their molecular weights i.e. small heat shock proteins, Hsp40, Hsp60. Hsp70, Hsp90, and Hsp100 in which Hsp70, Hsp40, Hsp27, Hsp60, and Hsp90 were found in Lewy bodies. Based on this observation, various in vivo and in vitro experiments were performed to confirm the presence. PC12 cell line along with 1-Methyl-4-phenyl pyridium(MPP+) was used as a PD model in an in vitro study. PC12 cell lines were heat shocked. The process was continued for 1 hour at 41.5°C. This treatment inhibited the cell death induction which was caused by a toxin when used for 6 hours before 1-Methyl-4-phenyl pyridium addition. In a different cell line, 1-Methyl-4-phenyl pyridium was seen to increase α-Syn mRNA expression causing leading to aggregation of the protein. In another study, heat shock proteins were found to have a protective action against toxicity caused by rotenone in the brain of rats. Heat shock also gave protection against apoptosis produced by α-Syn when human WT α-Syn or mutants A53T, A30P were expressed in *Saccharomyces cerevisiae.* α-synuclein expression in *Drosophila melanogaster* was seen to cause degeneration of Dopaminergic neurons in substantia nigra per compacta which could be improved by expression of Hsp70.[29]

Immunization

The current target of immunotherapy in Parkinson's disease is the ability of α-synuclein to transfer from one cell to another. Use of immunotherapy for α-synuclein is gaining a lot of interest. To move from cell to cell, α-synuclein has to enter the extracellular space. A lot of animal models have been used, trying to remove α-synuclein from this space and reduce the aggregation of the protein. Two strategies of immunotherapy have been tried in PD animal models: 1) Active immunization technique: antibodies are produced against α-synuclein by animal's individual immune system.[43] AFFIRis AG is an Austria based biotech company testing AFFITOPE® PD01A. It is a vaccine for α-synuclein which was assessed for 2 dose levels which were given once every month for 4 months to 24 PD patients and 8 healthy subjects and was found safe and was tolerated well. It developed α-synuclein antibodies in the serum of about 50% of the patients.[9] 2) Passive immunization technique: antibodies are directly administered against particular areas of α-synuclein.[43] Humanized antibodies for α-syn are being tested by Roche/ Prothena biosciences incorporative. In a study including forty healthy volunteers in phase, I trials the antibodies were proved safe and had a good tolerating power. It was able to reduce 96% α-synuclein level in serum.[9, 10]

Phosphorylation at Ser129

Sites for phosphorylation are found to be present in α-synuclein. It undergoes phosphorylation for various processes like LB formation, fibrillogenesis. Maximum of these processes occur at Ser129 in PD and other related diseases. The kinases responsible for this phosphorylation are CK1 and CK2, Sensitive factor attachment protein receptor 1, 2, 5 and 6, Dual specificity tyrosine-phosphorylation-regulated kinase1, Leucine-rich repeat kinase 2, PLKs 1, 2 and 3, human rhodopsin kinase-5. α-synuclein dephosphorylation is caused by protein 2 A. The phosphorylation studies have been supported by in vitro studies performed on murine and fruit flies. In these studies, phosphorylation of Ser129 was majorly responsible for the neurotoxicity caused by α-synuclein. [29]

Other molecules as α-synuclein aggregation inhibitors

Antibiotics have gained a lot of focus because of its multiple properties like anti-tumor and anti-inflammatory. It might possibly have a neuroprotective activity as well. After Rifampicin was found to decrease deposition and aggregation of β- amyloid, it was observed to have neuroprotective activity.

Ceftriaxone is another antibiotic which was also found to have neuroprotective activity. It binds to α-synuclein which does not allow its in vitro polymerization.[30]

Polyphenols which are known for its anti-oxidative property were found to have neuroprotective activity as well. Different polyphenols are investigated for their inhibitory action against α-synuclein fibrillation. Therefore they are being focused on modern PD studies.[31]

α-synuclein peptides are also used as α-synuclein aggregation inhibitors. Bodles and his co-workers in 2004 made N- methylated peptide by replacing Gyl73 with N-methyl glycine. This peptide along with other N- methylated peptides was used against α-synuclein aggregation as they are unable to form β- strands. α-synuclein peptides from a strong bond with the N- terminal of α-synuclein and inhibit its aggregation. [3]

ANIMAL MODELS FOR α-SYNUCLEIN AGGREGATION STUDY

Animal models used in the pharmacological evaluation of human diseases provide an overview of the therapeutic plan that can be used for the treatment of the diseases. Neurological diseases like PD can be studied using animal models. In PD, animal models have proven to be a successful approach. They can produce the same events and outcomes as in humans. Animal models to study the relationship between α-synuclein and PD are very limited in number. Some of these are discussed below:

- **Neurotoxin model**

Neurotoxins have been used find the role of α-synuclein in PD progression and pathogenesis. Recently, many studies are being performed using neurotoxins like MPTP and rotenone in rodents and in non-human primates. Using these models, the role of oxidative stress and other factors in alterations of the structure of α-synuclein and its aggregation was studied.[6]

MPTP (In mice)
For MPTP to be a reliable model in mice, stains like Swiss Webster mice and C57 should be used. α-synuclein aggregation is found in some models with chronic administration. Accordingly, on chronic treatment α-synuclein-ir was detected in dopaminergic neurons. When MPTP was given with probenecid, the α-synuclein-ir was found to contain protein covered lipid droplets during electron microscopy. These droplets were considered to be beneficial for LB development.[35] In another study, osmotic minipumps were used to administer MPTP chronically which developed α-Syn immune reactivity.[36]

MPTP (In non- human primates)
Using MPTP in non- human primates is an important PD model. Motor symptoms related to PD can be recreated using this model. MPTP can produce toxicity in the neurons due to which they cannot develop Lewy bodies but the unaffected neurons are found to have α-synuclein-ir.[33]

Pesticide model

Rotenone
Rotenone models make another successful model for PD and other synucleinopathies. Rotenone causes the loss of dopaminergic neurons in vitro. This occurs due to the inhibition of mitochondrial complex I causing an increase in ROS level leading to oxidative stress as shown in Figure 3 [37].

In an experiment, rotenone was injected to Lewis rats chronically. Rotenone due to its high mortality rate (approx. 30%) produced death of some animals but the remaining showed loss of dopaminergic neurons. The rats showed behavioral symptoms of PD. These animals were also observed to produce α-synuclein inclusion. Some of these had Lewy bodies more prominent then rest of the animal models.[38] It has also been found out that peripherally given rotenone causes aggregation of α-synuclein similar to PD. Rotenone is known to produce a change in the conformation of α-synuclein and leads the formation of fibrils. To reduce the high mortality rate, rotenone is given intra-cerebrally but it does not lead to the α-synuclein aggregation but instead causes neurodegeneration.[33].

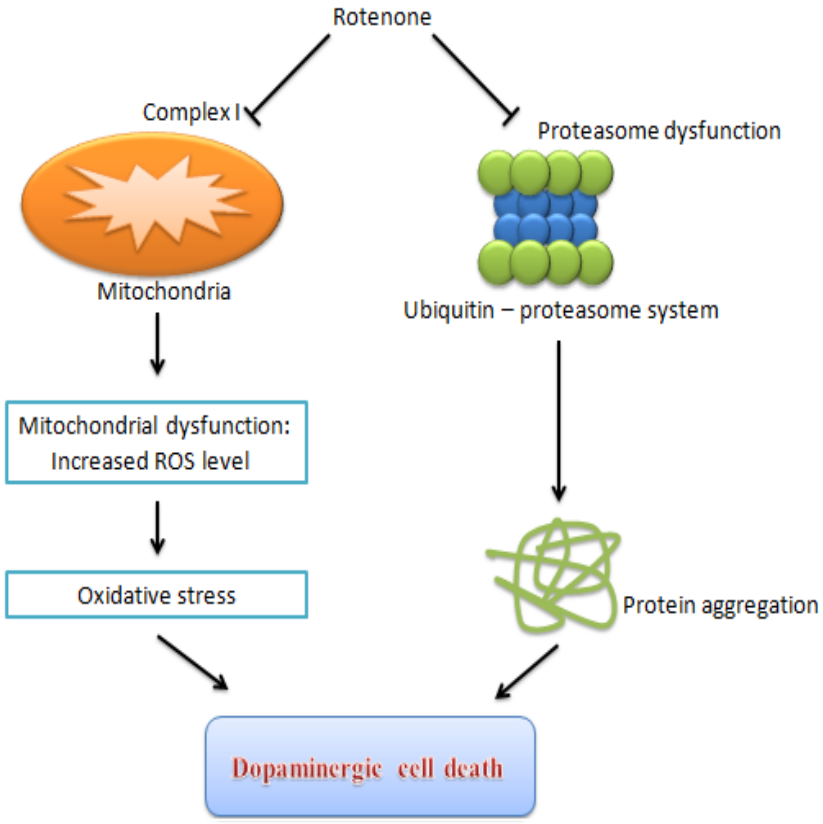

Figure 3 Mechanism of action of Rotenone

Genetic models

As discussed earlier, α-synuclein mutation in A53T and A30P links genetics to PD. These genes have a role in the production of transgenic mice. These genetically modified models have proved to be a huge success. It has shown that the mutation in A53T gene may lead to motor impairment or may even cause the death of the animal.

Drosophila models

This model shows the features similar to that of humans. It proves to be a good genetic method for PD. Drosophila melanogaster does not contain α-synuclein or its genes. Mutants A53T and A30P, as well as Wild- Type was overexpressed in Drosophila model in an experiment. Normal growth was seen in the neuronal cells of the flies though they began depletion of neurons after 30 days. Motor impairment was also observed in the flies. The flies with mutants and the wild- type both showed a disability to climb which started to begin within 23 days in A30P mutant *α-synuclein* due to its high toxicity. [39]

Transgenic models

Transgenic models are being widely used to study PD pathogenesis. Gene promoters are used to create these transgenic models. Mutation in SNCA gene produces LB like inclusions in mice but these inclusions only occur in A53T mutation. Using knockout α-synuclein to produce transgenic animals has no effect on the development of dopaminergic neurons and has no role in its degeneration.[34] Masliah E. et al., first produced transgenic mice by wild-type α-synuclein overexpression. They were found to have inclusion in different areas of the brain. The inclusions located in the PD brains of these mice had vacuole-like structure. The motor coordination of the animals with α-synuclein inclusion was found to be impaired on the Rotarod apparatus. However, no animal showed the loss of dopaminergic cells. [40]

Parkin gene rodent model

PD can also be generated by mutated parkin and Phosphatase and tensin homolog-PTEN-induced novel kinase 1 but no degeneration of dopaminergic cells have been reported in the knockout animals having the inclusion. The model

also failed to show any type of behavioral phenotype. It has been found out that neurodegeneration occurs in the mice with knocked out parkin in an elderly age. [34]

Leucine-rich repeat kinase 2 gene model

A major form of PD is known to be caused by Leucine-rich repeat kinase 2 gene mutation. Leucine-rich repeat kinase 2 is limited to the membrane. Although it has been known to have no effect on the development of dopaminergic neurons, the model shows a very low amount of neurodegeneration. [34]

Overexpression caused by vectors in rats

For the delivery of A30P α-synuclein to the SN of rats, Adeno- associated viruses (AAV) was used as a viral vector. α-synuclein aggregation was observed along with the loss of dopaminergic neurons (53%). The animals also showed rotations induced by amphetamine motor behavior was also seen to be impaired when compared the control group but these observations had no statistical evidence. [41] Another vector called Lentiviral vector was used to for the overexpression of A53T, A30P mutant human wild-type. This led to a loss of about 30% SN dopaminergic neurons. Recently, the used of vectors has also been tried on non-human primates which showed 30- 60% degeneration and loss of dopaminergic neurons in the SN. [42]

CONCLUSION

α-synuclein was discovered in the brain many years ago before its actual functions in the brain were discovered. The genetic link between α-synuclein and PD was an advancement in the disease. The study of the structure, propagation, and aggregation of α-synuclein has played a huge role in the treatment of the disease. Based on this, various therapies have been introduced including immunization against the disease. Many animal models are being used throughout the world to study the therapeutic techniques for the reduction in the synthesis, aggregation, propagation, and toxicity. Although more studies can be done to understand the physiological role of the protein as all the normal functions of α-synuclein is still unknown. This may help in making a progression in the study of PD.

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