The involvement of alpha-synuclein in Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is the second commonest neurodegenerative disease. Clinically, the disease is characterised by motor symptoms including resting tremor, and the loss of dopaminergic neurons in the substantia nigra. The histopathological hallmark of the disease is an inclusion body in the neuronal perikarya called Lewy body. The abnormal expression of alpha (α)-synuclein, a pre-synaptic neuronal protein, has been implicated as a biomarker of PD and involved in the pathogenesis of the disease. Amplification of the wild-type SNCA gene which encodes for α-synuclein, and its missense mutations lead to PD, thus implying that aberrant functions of the SNCA gene as well as its mutant expression are both capable of causing neurodegeneration. It is believed that α-synuclein contributes to the molecular pathological progression of PD in multiple ways. This article aims at reviewing the recent evidence pointing to the involvement of α-synuclein in the development of the neuropathological hallmarks associated with PD. By rationalising recent research findings on the aberrant expressions of α-synuclein, this review seeks to provide insights into the identified toxicities of this protein that may be targeted in the quest to design effective curative therapeutic interventions against the effects of the protein in PD.

1. Introduction

Aging poses a challenge to every living organism. In humans, the processes of cell division and metabolism occur energetically until the youthful ages, beyond which toxic byproducts of metabolism begin to accumulate [1], thus increasing the individual’s vulnerability to disease [2]. Human phenotypes of aging i.e. loss of function of tissues and/or organs [3] begin to appear, eventually leading to disease [1]. Among the several age-associated diseases, neurodegenerative diseases have being attracting enormous societal and scientific attention in recent times possible because of their irreversibility, lack of curative treatment strategies, and the numerous economic and social burdens they present [1].

PD is a chronic, age-dependent, progressive neurodegenerative disease [4,5]. The World Health Organisation (WHO) estimated that as of 2004, 5.4 million individuals were suffering from PD worldwide [6]. The disease manifestations are typically observed in people aged 65 years and above. PD causes several financial and social challenges, resulting from burdens such as; patients’ inability to work, high cost of drug therapy, ancillary care, medical visits, and hospital admissions. About US$ 23 billion is reportedly spent on PD annually in the United States of America [7]. A health economics study in the United Kingdom also estimated that about £6,000 is spent on caring for each PD patient yearly [8]. These burdens are predicted to correspondingly heighten in the future, indicating that the search for useful curative treatments remains a priority.

PD is the second commonest neurodegenerative disease [9] and the commonest neurodegenerative movement disorder [10]. Clinically, the disease is characterised by rigidity, bradykinesia, postural instability, resting tremor [10] and loss of dopaminergic neurons in the substantia nigra [11]. The histopathological hallmark of PD is an ubiquitin-containing cellular protein inclusion body in the neuronal perikarya called Lewy body (LB) [11]. Lewy neurites (LN), inclusion bodies identified in neuronal processes, have also been identified as a feature of the disease [11]. The 140-amino acid protein, α-synuclein, is implicated as a biomarker in PD pathogenesis, as it is a major component of both LB and LN [11]. Additionally, duplication and triplication of the
SNCA gene coding for α-synuclein[12,13], and missense mutations (A53T, A30P, E46K) in this gene[14-16] lead to genetic forms of PD, thus implying that overexpression of the wild-type α-synuclein and its mutant expression are both capable of causing neurodegeneration[17]. This review focuses on the molecular roles of α-synuclein in the development of PD.

1.1 α-synuclein and PD

1.1.2 α-synuclein

The SNCA gene codes for α-synuclein – a small, highly-charged polypeptide [18, 19]. α-synuclein is a soluble, heat-stable protein [19] mainly expressed in neuronal cells of the central nervous system, where it localises to pre-synaptic terminals closely associated with synaptic vesicles in physiological conditions [20]. Because the protein lacks a well-defined structure in aqueous solutions, it is sometimes referred to as a natively unfolded protein [18]. However, the protein forms α-helices on cell membranes upon binding to phospholipids and other lipids with net negative charges [18]. The heat-stable property of the protein is demonstrated on prolonged incubation where it forms β-pleated sheets [18].

α-synuclein consists of three different regions, namely: (a) an NH2 terminus consisting of the apolipoprotein lipid-binding motifs made of imperfect KTKEGV tandem repeats [19]. These repeats are believed to form the amphiphilic helices responsible for the protein’s tendency to form α-helices upon binding to plasma membrane [18]; (b) the mostly hydrophobic central region, also called the non-αI component (NAC) [18, 19]. This region confers the protein’s propensity to form aggregates and β-sheets [21]; and (c) a highly negatively-charged, acidic carboxyl terminus [19].

1.1.3 The synuclein protein family

α-synuclein belongs to the synuclein protein family, together with other β-synuclein and γ-synuclein. Whereas α-synuclein consists of 140 amino acids, β- and γ-synuclein are made up of 134 and 127 amino acids respectively [22]. Although all three family members are neuronal proteins that preferentially localise to pre-synaptic terminals in physiological conditions [20], α-synuclein is structurally distinct from the others in the NAC region [18, 23]. β-synuclein lacks the NAC region entirely, and γ-synuclein’s NAC component shares about 57% homology with that of α-synuclein [23]. It is suggested that α-synuclein aggregation is the major pathogenic hallmark of the disease, and that the protein’s NAC region is what propels its aggregation. This suggestion is also supported by the fact that neither the α-synuclein variants lacking the NAC region nor wild-type β-synuclein form cellular aggregates or confers neurotoxicity [21].

Although there are reports that link β-synuclein point mutations to rare cases of dementia with Lewy bodies [24, 25], the wild-type β-synuclein is said to offer neuroprotection against neurodegeneration mediated by its family member, α-synuclein, as described by [26, 27]. It is therefore appropriate to say that the wild-type β-synuclein protein may possess neuroprotective functions.

1.1.4 Putative biological function of α-synuclein

Since the Polymeropoulos et al., (1997) study [28] that implicated genetic defects in α-synuclein in familial PD, there have been several investigations into the normal function of α-synuclein and the causes of its neurotoxicity. α-synuclein is abundantly expressed in the nervous system [18]. In physiological conditions in neuronal cells, α-synuclein is present in pre-synaptic terminals, where it is closely associated with synaptic vesicles [18, 20].

According to [29], immunohistochemical studies of normal brains show that antibodies to α-synuclein give staining patterns of punctuate neuropil that are consistent with the staining pattern of pre-synaptic terminal. However, staining of neuronal soma were less noticeably observed in the normal brains studied. Interestingly, the expression of α-synuclein is not exclusive to neurons, because it is abundantly expressed in the circulatory system, mainly in the red blood cells [30], although its function in blood is quite unclear.

There is a suggestion that α-synuclein may function as a synaptic transmission regulator. Modulation of α-synuclein expression levels occurs in conditions that either confer injury or alter cellular plasticity [31, 32, 33]. It is observed how α-synuclein levels affect the release of the neurotransmitter catecholamine. In their studies, overexpressing both the wild-type and A30P mutant α-synuclein in PC12 cells blocked the release of catecholamine without affecting calcium threshold or co-operativity of catecholamine release. Although α-synuclein overexpression did not cause a reduction of vesicular pools, morphologically ‘docked’ vesicles were recorded in the overexpression cells, suggesting that aberrant α-synuclein inhibits secretory vesicle transport. However, neither the wild-type nor mutant α-synuclein overexpression affected vesicle fusion or catecholamine transmission. From the outcome of this study, it can be said that overexpression of α-synuclein blocks a particular initial step of vesicular transport, even though this blockage might occur before Ca2+-dependent vesicle membrane fusion. Other reports, such as those by [34-36] support the viewpoint that α-synuclein may act as a synaptic transmission modulator.

Fortinet al. [37] showed that fluorescently-labelled α-synuclein drifts away from vesicles during neuronal firing, but gradually returns thereafter. From this “drift-and-return” phenomenon, it
can be deduced that α-synuclein may be involved in cellular processes such as vesicle biosynthesis and transient binding to vesicles through its effects on phosphatidic acid metabolism [38]. In line with this suggested role of α-synuclein in synaptic transmission, the protein has been found to strongly regulate the mouse null phenotype for cysteine string protein-α (CSP-α), a presynaptic protein which shows synaptic degeneration [39]. In that study, it was argued that in a chaperone-like manner, α-synuclein synergises with CSP-α in assembling the SNARE complex, and that this may involve the protein’s binding to the membrane transport protein synaptotagmin-2 [40].

It can therefore be said that α-synuclein’s core function in neuronal cells may be the regulation of the release of neurotransmitters, through its effect on the SNARE complex. Probing these physiological functions of α-synuclein is therefore necessary in further understanding the protein’s role in PD.

1.1.5 α-synuclein aggregation

It is generally agreed that α-synuclein aggregation forms the basis of its neurotoxicity. On prolonged heating, both the wild-type α-synuclein and disease-associated point mutants have been shown to form aggregates [40], and that these aggregates are the major components of LB and LN recorded in PD [11, 18]. This discovery generates interest from scientists, as α-synuclein’s ability to form β-pleated sheets leads to the speculation that it might mediate a similar pathological process as the β-sheets of the β-amyloid protein which also forms fibrils in Alzheimer’s disease, thus pathologically unifying Alzheimer’s disease and PD.

1.1.6 α-synuclein fibrillogenesis

The aggregation process of α-synuclein into fibrils involves a cascade of events during which intermediates of the protein are formed, although the available molecular details are quite scanty. Initially, natively-unfolded α-synuclein monomers nucleate into soluble oligomers, which then metastabilise into protofibrils. Protofibrils are spherical-, string-like, soluble oligomeric derivatives of α-synuclein. Protofibrils then coalesce to form insoluble mature fibrils also called amyloid fibrils [41]. So in the aggregation process, the natively unfolded α-synuclein monomers, through the protofibril oligomer intermediate, form mature amyloid fibrils, which are pathogenically associated with PD.

From its kinetics profile, the aggregation process is said to be a rate-dependent process, with the rate-determining step being the formation of a nucleus of a critical number of α-synuclein monomers in specific conformations [42, 43]. Upon formation of these α-synuclein oligomers, the subsequent processes occur easily as they are thermodynamically favourable with negative Gibb’s free energy values.

A recent focus has been to identify which of the α-synuclein aggregation intermediates is directly associated with the observed neurotoxicity. This question has led to divergent observations. Whilst some argue that the α-synuclein oligomers or protofibrils cause the aggregation, other reports show that the mature fibrils are the culprits. Some of the more controversial viewpoints are given here. Dopamine, a monoamine neurotransmitter, and its metabolites inhibit protofibril conversion into mature fibrils both in vivo and in vitro, possibly by forming α-synuclein-dopamine adducts [44, 45] thus attributing the susceptibility of PD dopaminergic neurons to the upregulation in the formation of soluble oligomers. In a study in vitro cell free system, more protofibrils were formed by the A30P and A53T mutant α-synuclein than the wild-type, but the A53T mutant exclusively formed mature amyloid fibrils more readily than the A30P [41]. Yet the E46K mutant formed less protofibrils compared to the wild-type [46]. Therefore, no simple explanation can be given to the different tendencies in protofibril-formation between the wild-type and mutant forms of α-synuclein. Lashue et al., (2002) proposed a mechanism of action for protofibrils. They hypothesised that protofibrils create holes into vesicular membranes in order to permeabilise the membranes. This leads to the leakage of dopamine metabolites, which can eventually lead to oxidative stress [47]. In the defense of mature fibrils, it has again been suggested that mature fibril aggregates are inert and are also protective to protofibrils [48, 49]. Hence they cannot propel α-synuclein into form inclusion bodies. Another report also suggests that the existence of soluble oligomers, and not the mature aggregates, best correlates with neurotoxicity, as shown in vivo and in cellular systems [50]. This hypothesis has been explored to try and clear the toxic oligomers of α-synuclein. This study produced a considerable amount of success [51].

1.2 α-synuclein’s potential pathogenic effects

With the implied role of α-synuclein in PD, research continues to be keenly conducted into the pathogenic effects of the protein in such diseases. These studies have focused on cellular compartments and pathways such as the synapse, cytoskeleton, protein degradation system, the mitochondria, endoplasmic reticulum (ER)-Golgi vesicular transport pathway.

1.2.1 α-synuclein at the cytoskeleton

At the cytoskeleton, α-synuclein impacts on cytoskeletal dynamics, with evidence suggesting an interaction of the protein with microtubules. Whilst different research reports have described α-synuclein’s association with tubulin [52, 53], there are contradicting reports on the effect of α-synuclein on tubulin. Whereas some studies show that α-synuclein reduces tubulin polymerisation [54, 55], others point towards an enhancement
[56, 57]. Moreover, depolymerisation of tubulins is hypothesised to impair the maturation of prefibrils into amyloid fibrils in the fibrillogenesis pathway [58].

1.2.2 α-synuclein at the synapse

Results from in vivo and cell culture studies show that α-synuclein overexpression occurs in brains of autosomal dominant PD patients with SNCA genetic defects. This overexpression leads to consequences such as pre-synaptic protein loss, reduction in release of neurotransmitters, SNARE protein redistribution, synaptic vesicle enlargement, and synaptic vesicle recycle inhibition [59, 60].

1.2.3 α-synuclein on the protein quality control system

Another focal point is the effect of aberrant expression of α-synuclein on protein degradation. Investigations have shown that the expression of mutant α-synuclein and in some cases the wild-type protein impair the proteasomal degradation system [61-63], with this impairment creating a vicious loop of cellular accumulation of abnormally-folded α-synuclein. There exists the possibility that this accumulation occurs when proteasomal degradation of the α-synuclein soluble oligomers formed early in the fibrillogenesis pathway goes awry [64].

1.2.4 α-synuclein on the ER-Golgi vesicular pathway

Another cellular process possibly impaired by the aberrant expression of α-synuclein is the vesicular trafficking pathway. One of the early investigations conducted showed that fragmentation of Golgi bodies occurs early in PD pathology and that this fragmentation corresponds with the emergence of the protein’s soluble oligomeric forms [50]. A subsequent study reported that ER stress occurs early in neurotoxicity mediated by the A53T mutant. This study also showed that the observed cell death could be inhibited by a known ER stress inhibitor, emphasising that the ER-Golgi track perhaps is a principal target of α-synuclein. A number of studies have confirmed that the presence of α-synuclein in the mitochondria is capable of downregulating the activity of mitochondria complex I [73-77]. These studies did link the effects of mitochondrial α-synuclein with sporadic PD and mitochondrial toxins. Additionally, studies in transgenic mice show that α-synuclein disrupts brain mitochondrial morphology [80], and that the soluble oligomeric α-synuclein species are likely to trigger mitochondrial fragmentation, possibly leading to mitochondrial dysfunction and eventually cell death [81, 82]. Mutant α-synuclein also triggers mitophagy wrongly, which causes death of neuronal cells [83].

1.2.5 α-synuclein on the nuclear environment

Although α-synuclein has been reported to be apparently localised to the nucleus, observation of this localisation has been inconsistent [69, 70]. Studies in transgenic mice showed that phosphorylation of α-synuclein on Ser129 leads to the protein’s nuclear localisation [71]. It has been reported that α-synuclein associates with histones to decrease histone acetylation, and that histone deacetylase inhibitors rescue cells from α-synuclein-mediated cell death [72].

1.2.6 α-synuclein at the mitochondria: mitochondrial toxicity and oxidative stress

A number of studies have confirmed that the presence of α-synuclein in the mitochondria is capable of downregulating the activity of mitochondria complex I [73-77]. These studies did link the effects of mitochondrial α-synuclein with sporadic PD and mitochondrial toxins. Additionally, studies in transgenic mice show that α-synuclein disrupts brain mitochondrial morphology [80], and that the soluble oligomeric α-synuclein species are likely to trigger mitochondrial fragmentation, possibly leading to mitochondrial dysfunction and eventually cell death [81, 82]. Mutant α-synuclein also triggers mitophagy wrongly, which causes death of neuronal cells [83].

α-synuclein is shown to induce oxidative stress [84], through excessive production of reactive oxygen species (ROS), which causes the eventual death of neuronal cells, through the protein’s inhibition of mitochondrial complex I activity and activation of caspases. ROS, in the form of superoxide, and hydrogen peroxide, can be generated at the levels of complexes I to III in the electron transport system, and are catalysed to less harmful forms by mitochondrial antioxidants such as superoxide dismutase [85]. However, when the mitochondrial complex I is deficient, ROS levels increase beyond the mitochondrion’s ability to handle them. These mitochondria-derived ROS mediate apoptosis through mitochondrial DNA damage, oxidative damage of biological macromolecules, or the induction of cell death by the ROS directly. Research conducted in Drosophila [83, 85] and mammalian models of PD [86, 87] have provided evidence that overexpressing α-synuclein in duces ROS, and that suppression of ROS production do offer cellular protection.

2.0 CONCLUSION

α-synuclein serves as a useful PD therapeutic target. Further insightful studies towards deciphering the molecular mechanisms underpinning α-synuclein cytotoxicity is therefore key in the quest to develop appropriate novel treatments for the disease.
LIST OF ABBREVIATIONS

PD – Parkinson’s Disease; WHO – World Health Organisation; ROS – Reactive Oxygen Species; SNCA – Alpha-synuclein gene; LB – Lewy Bodies; LN – Lewy Neuritis; SNARE – Soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptors; ER – Endoplasmic Reticulum; PC12 – Pheochromocytoma 12 cell line; non-αβ component – NAC.

COMPETING INTERESTS

The author has no conflict of interest to declare.

Reference


