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Review

Toxicity of extracellular secreted alpha-synuclein: Its role in nitrosative stress and neurodegeneration

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ABSTRACT

It has been demonstrated that both oligomerisation and accumulation of α-synuclein (ASN) are the key molecular processes involved in the pathophysiology of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and other synucleinopathies. Alterations of ASN expression and impairment of its degradation can lead to the formation of intracellular deposits of this protein, called Lewy bodies. Overexpressed or misfolded ASN could be secreted to the extracellular space. Today the prion-like transmission of ASN oligomers to neighbouring cells is believed to be responsible for protein modification and propagation of neurodegeneration in the brain. It was presented that oxidative/nitrosative stress may play a key role in ASN secretion and spread of ASN pathology. Moreover, ASN-evoked protein oxidation, nitration and nitrosylation lead to disturbances in synaptic transmission and cell death. The interaction of secreted ASN with other amyloidogenic proteins and its involvement in irreversible mitochondrial disturbances and oxidative stress were also described. A better understanding of the mechanisms of ASN secretion and dysfunction may help to explain the molecular mechanisms of neurodegeneration and may be the basis for the development of novel therapeutic strategies.

1. Introduction

Since the discovery of α-synuclein (ASN) (Maroteaux et al., 1988), the last 20 years in the study of synucleinopathies have been abundant with exciting findings, novel hypotheses as well as controversial data. This time period could easily be divided into two periods: the first beginning after the publication of genetic data which showed that autosomal dominant point mutations of the ASN gene are associated with a familial form of Parkinson's disease (PD) (Polymeropoulos et al., 1997; Krüger et al., 1998; Zarranz et al., 2004). Concomitantly Spillantini et al. (1997) demonstrated the presence of ASN in Lewy bodies (LB), i.e. the intracellular inclusions found post mortem in PD brains. Moreover, ASN and its fragment, NAC (the non-amyloid-β component), were found in amyloid plaques associated with Alzheimer's disease (AD) (Ueda et al., 1993; Mikolaenko et al., 2005; Jellinger, 2004). All of these data provided strong evidence that ASN plays a key role in the pathogenesis of neurodegenerative diseases. The second period started after the discovery of ASN in the extracellular space, e.g. in blood plasma (El-agnaf et al., 2003) and in cerebrospinal fluid (CSF) (Borghi et al., 2000). Moreover, its oligomers were found in the CSF of PD patients (El-Agnaf et al., 2006). ASN was shown to be released from the nerve-ending fraction (Adamczyk et al., 2007), primary neurons (Emmanouilidou et al., 2010; Danzer et al., 2011) and other cells in culture (El-Agnaf et al., 2003; Sung et al., 2005; Lee et al., 2005). More recent evidence suggests that extracellularly liberated ASN oligomers play a key role in the progression of neurodegeneration (Lee, 2008; Brown, 2010) because they can be transferred between neurons (Desplats et al., 2009) or from neurons to glia (Lee et al., 2010), thus causing the death of the recipient cells. The presence of LB was discovered in grafted neurons in PD patients, thus suggesting that ASN-pathology can also propagate from host to graft (Li et al., 2008; Kordower et al., 2008; Chu and Kordower, 2009). In addition, stem cells or fetal tissue transplanted into the CNS of transgenic mice over-expressing human ASN showed intracellular deposits formed by host ASN (Desplats et al., 2009; Hansen et al., 2011). Moreover, it was demonstrated that ASN transferred between neurons can induce the aggregation of natively produced synuclein in recipient cells, which suggests that ASN possesses prion-like properties (Hansen et al., 2011). Although the hypothesis concerning the "prion-like"

Abbreviations: ·OH, hydroxyl radicals; AD, Alzheimer's disease; ASN, α-synuclein; Aβ, amyloid β; CaN, calcineurin; CREB, cAMP response element-binding protein; CSF, cerebrospinal fluid; DA, dopamine; DAQs, dopamine-quinones; H_2O_2, hydrogen peroxide; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; LB, Lewy bodies; MMPs, matrix metalloproteases; NAC, non-Aβ component of Alzheimer's disease; NF-κB, nuclear factor kappa B; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; O^{2-}, superoxide anion; $ONOO^-$, peroxynitrite; PARP-1, poly(ADP-ribose) polymerase-1; PD, Parkinson's disease; ROS, reactive oxygen species; TNTs, tunnelling nanotubes.

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spread of ASN gained the greatest interest, the mechanisms of its secretion and toxicity remain a puzzle until today. Given the importance of oxidative/nitrosative stress in neurodegenerative disorders, it is possible that free radicals may be an important factor involved in ASN dysfunction and release. In this review we integrate the current literature to show the importance of the crosstalk between oxidative stress and ASN spread in the pathogenesis of neurodegenerative disorders.

2. ASN secretion and transfer between cells: the involvement of oxidative stress

After the discovery of ASN in CSF and blood plasma, the question of the origin of extracellular ASN in body fluids arose. At first it was speculated that the uncontrolled and unspecific leakage of ASN might occur during disruption of the cell membrane in severe pathological conditions. However, this hypothesis was not sufficient in explaining the lack of differences between CSF levels of ASN in PD patients and in healthy individuals (Borghi et al., 2000). Furthermore, it was demonstrated that the release of ASN from cells does not coincide with the secretion of other small cytosolic proteins (Adamczyk et al., 2007; Lee et al., 2005). Secretion of ASN was also independent of the applied method of expression and the type of cellular experimental model (El-Agnaf et al., 2003; Sung et al., 2005; Lee et al., 2005). Taken together, these data suggest that the secretion of ASN is a highly regulated process occurring in normal, live cells; however, the contribution of cell death in ASN release cannot be ruled out entirely. Our own results indicated that extracellular liberation of ASN may be exacerbated by various oxidative stress conditions (Adamczyk et al., 2007). The mechanism of extracellular release of ASN has remained elusive until now. It is not clear whether the transport of either a monomeric or aggregated form of ASN is mediated by the vesicle-related exocytosis process (Lee et al., 2005; Jang et al., 2010); it was demonstrated that the classical endoplasmic reticulum/Golgi-dependent pathway is not involved in ASN secretion (Lee et al., 2005; Jang et al., 2010). Some data showed that oxidative stress is a key factor responsible for the elevated vesicular translocation and extracellular release of ASN (Adamczyk et al., 2007; Lee et al., 2005; Jang et al., 2010). Free radicals were shown to oxidise the tyrosine residues in the ASN structure into o,o′-dityrosine or 3-nitrotyrosine. Both modifications enhance the ability of ASN to misfold and to aggregate. The specific environment of the vesicular lumen, which additionally negatively influences protein folding, may further enhance its aggregation. It was previously shown that the intravesicular milieu with a high calcium concentration, low pH and the presence of glycosaminoglycans may accelerate ASN aggregation (Lowe et al., 2004; Hoyer et al., 2002; Cohlberg et al., 2002).

ASN was also shown to be secreted via the exosomes by a calcium-dependent mechanism (Lee et al., 2005; Emmanouilidou et al., 2010; Alvarez-Erviti et al., 2011b). Exosomes play an important role in intercellular communication by providing the exchange of RNA and proteins between the cells and by protecting their targets from cellular stress. They were shown to supply recipient cells with antioxidative resistance, although they play a protective role against oxidative stress and cell death (Eldh et al., 2010). It was documented that exosomes are involved in prion protein transmission from neuronal and non-neuronal infected cell lines to uninfected recipient cells (Alais et al., 2008; Rajendran et al., 2006; Fevrier et al., 2004; Vella et al., 2007). This process could also be involved in the propagation of ASN in a manner similar to prion diseases (Olanow and Prusiner, 2009). It is possible that the oxidative modifications of ASN may induce its aggregation and transmission to neighbouring cells and may initiate the vicious circle of prion protein spreading and oxidative stress.

It was suggested that the intercellular transfer of ASN could also occur between neighbouring neurons via tunnelling nanotubes (TNTs) (Gousset et al., 2009). These TNTs are long, thin extensions comprising F-actin, between 50 and 200 nm in diameter. Direct cell-to-cell transmission via TNTs could mediate the spread of ASN and explain how neuropathology in PD spreads in accordance with Braak's concept, which suggests that there is a progression of LB pathology from the brainstem to the cortex along specific neural pathways (Braak et al., 2003).

Extracellular ASN is removed either by proteolytic degradation by proteases or by uptake into neighbouring cells. Previous in vitro data identified two enzymes responsible for the cleavage of extracellular ASN. One of them is neurosin, a serine protease which is predominantly expressed in the central nervous system (Tatebe et al., 2010). This enzyme was shown to cleave the ASN protein predominantly within its central part, thereby hampering its aggregation and toxicity. As both phosphorylated and mutant ASN (A30P) were shown to be more resistant to neurosin degradation, it was postulated that in PD-related pathological conditions the insufficient or abnormal extracellular cleavage of ASN might cause its dangerous spread into recipient cells (Kasai et al., 2008). Extracellular ASN could also be efficiently degraded by matrix metalloproteases (MMPs), especially MMP 3 (Sung et al., 2005). This enzyme degrades ASN from its C-terminal and has at least four cleavage sites within the NAC sequence, thereby producing NAC-like smaller fragments that have been shown to increase ASN aggregation. Interestingly, activation of the proteolytic fragmentation of ASN by MMPs was significantly increased by nitric oxide (NO) (Sung et al., 2005). Recently, it was found that plasmin cleaved and degraded extracellular ASN specifically in a dose- and time-dependent manner. Plasmin cleaved mainly the N-terminal region of ASN and also inhibited the translocation of extracellular ASN into the neighbouring cells in addition to the activation of microglia and astrocytes by extracellular ASN (Kim et al., 2012).

3. The role of oxidative stress and nitric oxide (NO) in ASN toxicity

The cytotoxic effect of extracellular ASN and its hydrophobic fragment, the NAC peptide, were first reported in neuroblastoma SH-SY5Y cells (El-Agnaf et al., 1998) and the B12 cell line (Liu and Schubert, 1998). Many consecutive in vitro studies confirmed these results and demonstrated the toxic effect of either fibrillar aggregates (Bodles et al., 2000) or the monomeric/oligomeric species of ASN (Du et al., 2003; Kazmierczak et al., 2008; Adamczyk et al., 2009). The effect of extracellular ASN on neuronal cell function is still not fully understood.

One of the mechanisms of ASN neurotoxicity is the enhancement of the reactive oxygen and nitrogen species level. It was previously shown that the accumulation of ASN is associated with oxidative modifications of proteins and lipids (Bossy-Wetzel et al., 2004; Lin and Beal, 2006), thus leading to macromolecule damage and neurodegeneration. Exogenous ASN was shown to increase NO synthesis. Our study reported that ASN added extracellularly induced NMDA receptor-mediated activation of neuronal nitric oxide synthase (nNOS) and increased the NO level in the rat brain (Adamczyk et al., 2009). Since both competitive and non-competitive antagonists of the NMDA receptor prevented ASN-evoked NOS activation (Adamczyk et al., 2009), it seemed possible that ASN could directly interact with the NMDA receptor and change its structure and sensitivity to glutamate or to its co-factor, D-glycine. Previous findings indicated that ASN also induces the Ca^{2+} influx via activation of N-type voltage-dependent calcium channels (Adamczyk and Strosznajder, 2006) or may stimulate the Ca^{2+} influx directly by forming pore-like annular structures in