Will Preventing Protein Aggregates Live Up to Its Promise as Prophylaxis Against Neurodegenerative Diseases?

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Protein aggregation and misfolding characterize most age-related neurodegenerative diseases including Alzheimer, Parkinson and Huntington diseases. Protein aggregation has generally been assumed to be responsible for neurodegeneration in these disorders due to association and genetics. However, protein aggregation may, in fact, be an attempt to protect neurons from the stress resulting from the disease etiology. In this review, we weigh the evidence of whether removal of amyloids, aggregates and neuronal inclusions represent a reasonable strategy for protecting neurons.

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Introduction

Highly insoluble protein aggregates, within and outside the plasma membrane, are an invariant feature of a number of neurodegenerative diseases. Amyloid-β (Aβ), senile plaques, and intracellular neurofibrillary tangles (NFT) containing tau protein occur in Alzheimer disease (AD) (97); Lewy bodies of Parkinson disease (PD) contain fibrillar α-synuclein; prion disease contains prion aggregates; Huntington disease (HD) contains huntingtin aggregates. The central hypothesis in most neurodegenerative disease research is that such protein aggregates, specific to each disease, are the major cause of neurodegeneration (105). This idea is supported by the fact that protein aggregates, such as Aβ and α-synuclein, have been shown to kill neurons in vitro (77, 113, 115) and therefore, it is assumed they must be responsible for the damage observed in the affected human brain. The increasingly sophisticated approaches being developed and applied to the removal of protein aggregates lead us to remember Hawthorne's admonition of almost two centuries ago (37). In a story questioning the arrogance of the approaches of science, he considers a scientist who marries a beautiful woman with one defect, a birthmark. His concerted efforts to remove the "ugly" blemish are successful but with them, he loses the part of her that was essential to her humanity and she dies. Might we again be looking at "birthmarks" in these protein aggregates, when efforts to remove Aβ have hastened the demise of patients? In contrast to the view expressed above, we have argued for the innocence of Aβ, thinking instead that Aβ is a much maligned protector of the brain (85), and here we extend our arguments to the aggregations found in other neurodegenerative diseases such as PD and HD.

The Ugly?

AD, PD, HD, Pick disease, and motor neuron disorders have been suggested to be caused by protein aggregation.

Alzheimer disease. Study of the brain in cases of AD demonstrates that the Aβ peptide is the major constituent in 2 of the hallmark pathologies, namely senile plaques and cerebral amyloid angiopathy (28, 29, 60). Aβ is derived by proteolytic cleavage from the amyloid-β protein precursor (AβPP), a protein with multiple cellular functions that has the general properties of a cell surface receptor (47, 68). AβPP was first identified as a transmembrane protein in the neuronal plasma membrane, but in a more recent study, it was shown that the protein is also processed into a secreted form in the Golgi apparatus before it ever reaches the cell surface (11, 15, 52). Aβ was originally thought to be an abnormal cleavage product. However, Aβ has since been established as a normal metabolic product of neuronal AβPP and is found in the cerebrospinal fluid (CSF) and serum of healthy individuals (33, 94). AβPP metabolism is regulated by 3 different proteolytic enzymes, α, β and γ-secretases, at their specific cleavage sites, which yields a number of different products, including $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ (96). While $A\beta_{1\text{-}40}$ is the predominant product of this proteolytic pathway, $A\beta_{1\text{-}42}$ is far more fibrillogenic in vitro and is the major Aβ species present in the core of senile plaques (both AD and non-AD related plaques) (8, 43). The deposition of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ into senile plaques likely

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begins with the nucleation of soluble $A\beta_{1-42}$ into fibrils followed by recruitment of normally soluble $A\beta_{1-40}$ (43). Microenvironmental changes in the brain, such as pH, metal ion availability and oxidants likely impact Aβ conformation and its subsequent deposition into senile plaques (1, 98). Recently, a great deal of attention has also been focused on the fact that soluble forms of Aβ, that are "prefibrillar," may be involved in the pathogenesis of AD (53).

Parkinson disease. Protein aggregation in dopaminergic neurons of the substantia nigra is the most common pathology associated with neurodegeneration in PD. For instance, α-synuclein and Parkin are present in Lewy bodies as dense filamentous aggregates in sporadic PD (13, 65). Accumulation of α-synuclein in cultured human cells also causes selective degeneration of dopaminergic neurons in the presence of dopamine, but not in non-dopaminergic neurons, suggesting a selective toxicity that is dependent upon accumulation of aggregates (113). In addition, mice expressing the human Ala53Thr α-synuclein mutation exhibit adult onset neurodegeneration and α-synuclein aggregation in the brain (54). Moreover, mutations in either α-synuclein or Parkin are associated with inherited PD (90). Parkin is similar to the ubiquitin family of proteins, which is associated with the pathogenesis of several neurodegenerative diseases and is a component of Lewy bodies in PD (42) as well as of NFT in AD (64). Parkin is a protein-ubiquitin E3 ligase (93) and a variety of mutations exist in the Parkin gene of patients with autosomal recessive (55) or sporadic PD (88). Many of these mutations appear to be clustered in the ubiquitin-like domain and the RING (Really Interesting New Gene) finger domains (27), suggesting that the E3 ligase activity of Parkin is crucial for preventing PD. It was also shown that Parkin is bound to α-synuclein in conditions associated with α-synuclein aggregation (13, 87). Furthermore, Parkin has been found to be upregulated during the coordinated cellular response to unfolded protein-induced stress (41).

Huntington disease. The conformational change and aggregation of huntingtin is thought to be responsible for neurodegeneration in HD (3). HD is an autosomal dominant genetic disorder and the underlying genetic defect is expansion of an unstable DNA segment, which contains the polymorphic trinucleotide CAG repeat. The coding sequence of the IT15 gene is located on chromosome 4, which encodes the highly conserved protein, huntingtin (40) and expanded CAG trinucleotide repeats are linked to the development of neurologic disease (83). However, while polyglutamine expansion appears to be a common element in several genetic disorders that may lead to a toxic gain of function, the underlying mechanism of its toxicity in HD remains unknown. Recent evidence suggests that the selective neuronal resistance or vulnerability to the degenerative process in HD may depend on the intrinsic level of normal huntingtin expression in either unaffected or affected cell types and their regional distribution (22). In transgenic mouse and cell culture studies, the expanded CAG repeat of huntingtin was shown to result in intranuclear and cytosolic aggregates and dystrophic neurites (16, 19). Although it is unclear what role huntingtin aggregation plays in the pathogenesis of HD, it has been hypothesized that the inclusions are formed by the aggregated N-terminal truncation product of huntingtin causing neuronal death through alterations in nuclear transport, or by affecting chromatin structure affecting transcription (19, 83).

The Beautiful?

Alzheimer disease. Investigators studying the primary culprit associated with AD have, as highlighted above, primarily focused on Aβ such that the *amyloid hypothesis* (Figure 1A) is the predominant mechanism thought to be responsible for the disease (35). However, a number of important caveats have led us to seriously question the validity of this hypothesis (76, 85). First, the temporal occurrence of amyloid during disease development argues against its importance. In cell culture "models," Aβ can lead to oxidative stress, yet it is apparent from cell (23, 114), animal (21, 79) and human (70, 72) studies that oxidative stress temporally precedes Aβ deposition. Moreover, such oxidation-mediated increases in Aβ are associated with a decrease in oxidative stress indicating a potential antioxidant action of Aβ (Figure 1B) (71, 72). Therefore, the early interpretations based on the cell culture "models," which were instrumental in formulating the *amyloid hypothesis*, are clearly neither an accurate reflection of in vivo nor diseased conditions, and simply reflect artifactual cell culture conditions (84) that fail to provide insights on the role of Aβ in vivo (85). Additionally, it should be noted that the deposition of Aβ into senile plaques is by no means specific to AD patients and, in fact, seems to be a part of normal aging (18). The incidence of senile plaques in control individuals increases with age, as does the incidence of AD, and the number of senile plaques in cognitively normal individuals can rival that

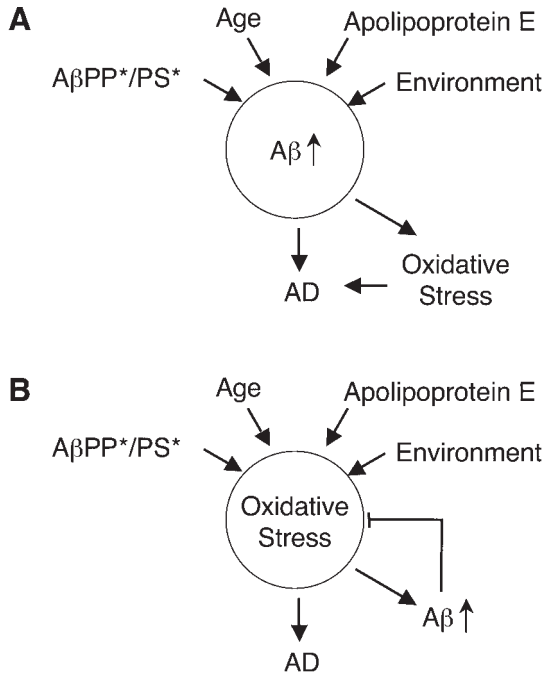

Figure 1. A. The *amyloid hypothesis* proposes that all risk factors for disease, sporadic as well as genetic through mutations (AβPP*/PS*), lead to increases in Aβ that then directly leads to oxidative stress and AD. **B.** Our *alternate hypothesis* proposes that these same risk factors for disease lead to increased oxidative stress that then leads to increases in Aβ and AD. Further, such oxidatively-induced Aβ actually serves to attenuate oxidative stress by an antioxidant action.

found in patients with advanced disease (57). Even considering only those patients with AD, there is a weak correlation between the burden of Aβ and neuronal loss or cognitive impairment (18, 67). Moreover, increased production and deposition of Aβ in the central nervous system is observed in response to injury, including ischemia and head trauma (25, 26, 81). Also, despite marked Aβ deposition in the brains of Down syndrome patients by the second decade of life, there is little evidence of further cognitive decline until advanced age. Could it instead be that Aβ is a protective cellular factor necessary to maintain homeostatic balance from the insults that cause AD (45, 99)? Recent reports support the view that Aβ peptides, which are normal metabolic products, have a protective effect; in some conditions, they may function as either an antioxidant (39, 120), a trap or sink (82). In this light, Aβ would likely serve an analogous function in the brain to that of albumin in the systemic circulation, which binds metals, drugs, metabolites, and proteins (50). These findings are consistent with the trophic and neuroprotective action of Aβ at physiological concentrations under serum-deprived culture conditions and in neonatal cells (4, 46, 49, 56, 78, 95, 101, 103, 110, 111, 115).

Second, if, as we suggest, Aβ is protective, how can we explain the Aβ toxicity demonstrated in in vitro studies? As mentioned above, while Aβ can be toxic for animal or human cells in culture, it does so only at high concentrations. One can infer significance for Aβ's LD50 like one can for any compound, for even NaCl and H_2O are toxic in excess and must be viewed in the context of in vivo concentrations and environment. In brain there is generally little cell death associated with Aβ deposition (57) and, consequently, only a weak correlation with cognitive decline (67). Additionally, neuronal cells in culture actively grow on isolated AD plaques unless the plaques are modified by microglial cells (20). Overall, Aβ deposition appears correlated best with responses to neuronal injury (26), of which the most pronounced and chronic is aging, rather than being the mediator of such an injury. This scenario is consistent with a protective function for Aβ (2, 45, 85). We suspect that the underlying stress in AD is energetic, since a depletion of the energy supply induces upregulation of AβPP expression such that ischemia, hypoglycemia and traumatic brain injury, a condition that has been shown to put neurons under metabolic stress (112), all upregulate AβPP and its mRNA in animal models and cell culture systems (34, 44, 66, 91, 92, 116). Not only does energy shortage and Ca^{2+} dysregulation promote AβPP expression, but they also route the metabolism of AβPP from the non-amyloidogenic to the amyloidogenic pathway. Inhibition of mitochondrial energy production alters the processing of AβPP to generate amyloidogenic derivatives (23, 24, 62), while oxidative stress has been specifically shown to increase the generation of Aβ (23, 63, 75). Consistent with this response, Aβ has been detected in the human brain several days after traumatic brain injury (26). This fits well with the role of AβPP as an acute phase reactant upregulated in neurons, astrocytes and microglial cells in response to inflammation and a multitude of associated cellular stresses including axonal injury (6, 26), loss of innervation (108), excitotoxic stress (74, 104), heat shock (14), oxidative stress (23, 114), aging (38, 69, 106) and inflammatory processes (7). Other pro-inflammatory stimuli that mediate the synthesis and release of AβPP include IL-1β (9, 30) and TNFα converting enzyme (10). The increased expression of AβPP under

these stress conditions is likely a reaction to decreased energy supply.

The strongest evidence supporting the Aβ hypothesis of AD is provided by familial forms of the disease, which involve a mutation in AβPP, or polymorphisms in genes that are directly involved in AβPP processing (35, 89). In the past decade, a tremendous amount of effort and resources have been dedicated to determining the pathological mechanism underlying AD using models based on these mutations. However, mutations in AβPP have been identified in only 20 to 30 families worldwide and represent less than 0.1% of the 15 million known cases of AD; mutations in both presenilin (PS) 1 and 2 account for only an additional 120 to 130 affected families. It is clear that mutations in these proteins affect AβPP processing and are capable of inducing amyloid deposition and dementia. The key question is, however, whether the link between the mutations and disease development is direct or indirect (Figure 2). Indeed, since the sensitivity of the neuronal environment to insults increases with advancing age, it is very likely that the most important parameter in the development of AD involves mechanisms, ie, oxidative stress, that are strongly associated with aging. In this regard, it is noteworthy that no aberrant neuropathologies are observed prior to the onset of dementia in middle age. Therefore, we think Aβ aggregation and amyloid deposition may be viewed as either the savior of neurons from devastating conditions, or the result of a cellular response to protect neurons from injury (Figure 1B).

That Aβ and senile plaques might represent a protective adaptation to preserve homeostasis begs the question of whether a similar attribute might be ascribed to the tau protein and neurofibrillary tangles. In this regard, there is significant evidence that tau phosphorylation is controlled by oxidative stress (102, 117-119) and consequently serves as an oxidative sink (12, 31, 109) that reduces oxidative damage to key macromolecules (70, 72). Therefore, we suspect that tau, like Aβ, is serving a protective antioxidant function in the aging and diseased brain (100).

Parkinson and Huntington diseases. As in AD, the easy assumption is that the aggregation of disease specific proteins is a major cause of PD and HD. However, we propose that the protein aggregates associated with PD and HD have a protective function in response to primary insult.

Again, as for Aβ, aggregation of α-synuclein in vitro was shown to result in the selective degeneration of dopaminergic neurons (54, 113). However, the toxic

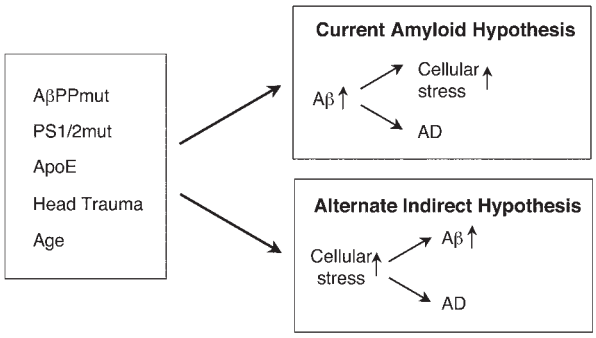

Figure 2. The current *amyloid hypothesis* supposes that the mutations in AβPP and PS (AβPPmut / PS1/2mut), as well as other risk factors, affect AβPP processing, leads to amyloid deposition and this increase in Aβ causes AD. We propose an *alternate indirect hypothesis,* which proposes that these mutations, as well as other risk factors, lead to increased cellular stress that then leads to AD as well as a disease-independent increase in Aβ.

mechanism by which this occurs remains relatively obscure, and other studies using different models failed to show consistent neurotoxicity by α-synuclein (36, 59, 61, 73). Furthermore, there are several lines of evidence suggesting that α-synuclein may, like Aβ, play a protective role (36, 58). For example, the oxidative stress caused by the herbicide paraquat causes α-synuclein aggregation in experimental animal brain and this increased expression and aggregation of α-synuclein was shown to be neuroprotective (58). Given that many different types of neurotoxins such as MPTP and rotenone increase α-synuclein expression in brain (5, 107), it is quite possible that the increase of α-synuclein represents an adaptive response to toxic stimuli. Indeed, overexpression of α-synuclein in transgenic mice does not consistently result in neuronal damage (59, 61), nor does it exacerbate neurodegeneration caused by MPTP (80). Therefore, α-synuclein itself may possess properties that counteract toxic injury, and its expression could be associated with cell survival strategies as suggested by Manning-Bog and colleagues (58).

The other constituent of the Lewy body in PD is Parkin. As with α-synuclein, the expression of Parkin is also known to be regulated by cellular stress. Parkin is upregulated as part of the unfolded protein response and specifically blocks unfolded protein stress-induced cell death (41). The mechanism of neuroprotection by Parkin is thought to be mediated through ubiquitin-proteasome mediated protein degradation. This view is supported by the observation that the protective effect of Parkin is significantly reduced by treatment with lactacystin, a potent proteasome inhibitor (17).

We view the role of huntingtin and its aggregation in HD in a similar way, since the emerging data supports a neuroprotective role for it. In a postmortem study, it was reported that the immunocytochemical distribution of the N-terminus of huntingtin nuclear aggregates in HD does not correspond with neuropathology (32, 51). In addition, the nuclear aggregation of huntingtin is not required for the initiation of neurodegeneration, nor is it a predictor of neuronal death (48, 86). In primary neuronal cultures, Saudou and colleagues (86) found no correlation between the presence of nuclear inclusions and mutant huntingtin-induced death. This implies that aggregation may sequester mutant huntingtin and play a protective role in the disease. The comparisons to the scheme depicted in Figure 2 are compelling. Thus, aggregation, particularly nuclear aggregation, may be of less importance to the cascade of events leading to neuronal cell death in HD than was previously implied. Rather than being a harbinger of neuronal death, huntingtin aggregation may be a cytoprotective mechanism that inactivates polyglutamine-induced neurotoxicity.

Does Removal of the Ugly Create Beauty?

Are amyloids, aggregates, and neuronal inclusions toxic to neurons? Are we focusing on them merely because they, as birthmarks (or better stated, age marks), are ugly? If so, as in the case of the beautiful wife, will we lose our humanity by neglecting to realize that we need as much the ugly as the beautiful? It is the balance of these and so much else that defines a healthy life. While we by no means intend to suggest that protein aggregates play a negligible role in disease pathogenesis or that modulating them may have some clinical benefits, it is very unlikely that they are the proximal mediator of neurodegeneration. As detailed above, many lines of evidence exist to support an alternate role for them in the neurodegenerative process. It may be premature to determine that they are saviors of neurons, although such a statement would not be without evidence. Nonetheless, we hope that by opening up the weaknesses that challenge "dogma," we can clarify that the "ugly" has little to do with initiating disease and therapeutic focus on removing the "devil" will have little efficacy.

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