

Oxidation and ubiquitination in neurodegeneration

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Abstract

It is widely accepted that protein oxidation is involved in a variety of diseases, including neurodegenerative diseases. Especially during aging, a reduction in anti-oxidant defence mechanisms leads to an increased formation of free radical oxygen species and consequently results in a damage of proteins, including mitochondrial and synaptic ones. Even those proteins involved in repair and protein clearance via the ubiquitin proteasome and lysosomal system are subject to damage and show a reduced function. Here, we will discuss a variety of mechanisms and provide examples where cognition is affected and where repair mechanisms are no longer sufficient to compensate for a dysfunction of damaged proteins or even may become toxic. Next to physiological deficits, an accumulation of deficient proteins in aggresomes may occur and result in a formation of pathological hallmark structures typical for aging and disease. A major challenge is how to prevent aberrant oxidation, given that oxidation plays an essential role in aging and neurodegenerative diseases. Particularly interesting are the possibilities to reduce the formation of radical oxygen species leading to a dysfunction of protein repair and protein clearance, or to a formation of toxic byproducts accelerating neurodegeneration.

Keywords: Alzheimer's disease, oxidation, mitochondria, neurodegeneration, tau protein, ubiquitination

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Introduction

Oxygen plays an important role for most living organisms, with benefic and also adverse effects. Reactive oxygen species (ROS), i.e. unpaired electrons or free radicals, are produced by a variety of mechanisms^{1,2} and often are byproducts of metabolic processes that lead to oxidation, peroxidation, glycation or nitrosylation. Most biological systems have developed different strategies how to neutralize free radicals, i.e. by different enzymes such as glutathione, thioredoxins, peroxiredoxins, reductases, phosphatases and kinases involved in redox regulation or transcription factors of the *Jun* and *Fos* families or NF- κ B.³ The cell has also repair mechanisms in place, which involve chaperones and heat-shock proteins to reverse conformational changes and, if not possible, to activate enzymatic clearance mechanisms via the ubiquitin proteasome system (UPS) or via lysosome-autophagy. This may eventually create problems when proteins cannot be repaired or degraded and then become toxic for the metabolism.⁴ There are a variety of strategies how to affect the dangerous development and attack noxious effects.

With aging, the formation of ROS is increasing, becoming more important for age-related diseases, and may result in oxidative damage, favouring the progression of many

pathologies.⁵ Therefore, it seems essential to look for strategies that tackle the problem early on during oxidative stress. In this minireview, we will look at critical and common points between different diseases regarding oxidation and the role of ubiquitination. Although some of the deficient mechanisms are not directly at the origin of the disease, they may affect the progression of the pathology and provide potential therapeutic targets.

Role of oxidation in aging, mitochondrial and synaptic defects

Normal brain aging is characterized by cognitive decline, with a moderate neuron loss and synaptic changes.^{6–8} Increasing evidence suggests that synaptic mitochondrial dysfunction is strongly associated with synaptic failure in many neurodegenerative diseases and represents an early event in neurodegenerative diseases such as Alzheimer's disease (AD).^{9,10} Synaptic mitochondria undergo multiple malfunctions including A β accumulation, increased oxidative stress, decreased respiration and compromised calcium handling.¹¹ The microtubule-associated tau protein probably plays a crucial role, since tau oligomers may impair memory and induce synaptic and mitochondrial dysfunction and neurodegeneration in mouse models.^{12,13}

Tau seems instrumental and depends on a variety of modification such as phosphorylation, acetylation, glycosylation and ubiquitination and, together with A β peptides, to have noxious effects on neurons.^{14,15} Oxidation may affect specific receptors and disturb the N-methyl-D-aspartate (NMDA) receptor excitation and inhibition balance.¹⁶ Such receptors comprise cysteine switches and are a molecular model for the action of S-nitrosylation,¹⁷ therefore sensitive to oxidation. Redox modulation by S-nitrosylation may also contribute to protein misfolding, changes in mitochondria dynamics and neuronal damage in neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and may be at the origin of foldopathies.¹⁸ Oxidative stress and consequences of oxidation are at the origin of many aging-related and pathogenic mechanisms which have been extensively discussed.¹⁹ A variety of cells are involved in these events such as glia, microglia and neurons, and mechanisms include mitochondrial impairment in synapses, the generation of free radical oxygen species and an increase in cell toxicity. This may have an effect on energy transfer mechanisms and stimulate microgliosis and neuroinflammatory events via cytokines. Cytoskeletal abnormalities may result in an alteration of axonal and dendritic transport and in an accumulation of aggregates. A general increase in oxidative stress leads to mitochondrial failure, to an activation of excitotoxicity and caspase cascades, to DNA damage and to an increased vulnerability of neurons for neurodegeneration.¹⁹ Increased oxidation affects protein conformation, therefore leading to dysfunction. Misfolding of proteins, summarized as *foldopathies*, is also known as proteinopathies or conformational diseases. They comprise clinically and pathologically diverse disorders in which specific proteins accumulate in cells and tissues, and are found as aggregates or inclusion bodies.^{20,21} They include over 40 diseases, including Alzheimer's, Parkinson's and Huntington disease, amyotrophic lateral sclerosis (ALS), prion disease, inclusion body myopathy and systemic amyloidosis. Advanced age is an important risk factor for most proteinopathies, in parts because the ability to degrade or remove abnormal proteins by ubiquitination and the proteasome are compromised. In several neurodegenerative diseases, tau and α -synuclein proteins play a role and may be summarised as *tauopathies* or *synucleinopathies*. A variety of proteomic studies have clearly identified that during aging mitochondrial dysfunction and free radical accumulation may lead to increased oxidation and dysfunction in protein degradation.^{20–24}

Lipofuscin, also called dark fat, aging pigment or ceroid, is of yellow-brown appearance. It is a 'pathologically derived storage material' composed by two-thirds of proteins and one-thirds of lipids, containing also iron and copper. It is detectable across a broad spectrum by fluorescence microscopy (540–650 nm). Free radicals influence senescence of mitochondria and reduce lysosome function. Furthermore, a dysfunction of the proteasome may contribute to an accumulation due to an incomplete elimination of cellular 'garbage'. Accumulation of ceroids represents a consequence of failed degradation of mitochondria or can be due to mutations in enzymes of the lysosomal system.²⁵

Since oxidative stress influences early pathogenic mechanisms involved in neurodegeneration, it is among the main targets in therapeutic strategies to increase oxidative defence mechanisms. So far, anti-oxidant treatments in clinical trials showed modest positive effects in cognitive function, possibly related to the fact that either anti-oxidants might not cross the blood-brain barrier, or that late-stage AD patients were used.^{11,26} There are a variety of therapeutic options.^{27,28} To be effective to prevent ROS formation, one needs to start early with a treatment. It is well known that certain products and food components have a positive effect on mental health and slow down cognitive decline with aging²⁹ by affecting oxidative stress, inflammation, atherosclerosis, cancer formation and much more, as reviewed recently.^{3,30} Therefore, diet may be a non-negligible factor to affect the progression of neurodegenerative disorders. However, in Huntington's disease, a neurodegenerative disorder that is characterized by polyglutamine aggregation, the anti-oxidant curcumin had rather an opposite effect and induced expansion of polyglutamine chains, aggregation of huntingtin, with a proteasomal dysfunction and eventually led to cell death.³¹ This suggests that a variety of mechanisms involved may react in different ways to anti-oxidant substances.

Dysregulation of protein repair and proteasome

The UPS is instrumental for protein repair and turnover and consists of a complex multistep enzymatic pathway, being a special challenge for the postmitotic neurons to maintain synaptic plasticity and self-renewal.^{4,32,33} Ubiquitin is a small molecule used to target damaged proteins for repair or proteins destined for degradation. The UPS is responsible for breakdown of 90% of abnormal proteins or short-lived proteins (t1/2 hours to days).³⁴

Oxidised and misfolded proteins may be repaired by several heatshock proteins (hsp27, hsp70, carboxyl terminus of the hsc-70-interacting protein, also called CHIP),^{35,36} and a repair is essential since several proteins may exert at least at some stage an 'ad hoc' toxic effect.³⁷ In a variety of diseases, a genetic variation leads to the expression of mutant proteins, such proteins being able to overload the UPS and consequently accumulate in form of protein aggregates or aggresomes as a result of ligase deficiency in the process of proteasome function.³³ In ALS, a fatal motor neuron disease, neurofilament proteins accumulate in the soma of motoneurons and superoxide dismutase 1 (SOD1)-mediated toxicity seems at the origin.³⁸ In Huntington disease, a huntingtin mutation results in polyglutamine accumulation. In Parkinson's disease, there is a mutated parkin (an E3 ubiquitin ligase) and α -synuclein as substrate that accumulate in Lewy bodies. A recent review illustrates the role of oxidation of dopamine to aminochrome (a precursor of the neuromelanin) that induces (i) the formation of α -synuclein protofibrils that inactivate chaperone-mediated autophagy; (ii) the formation of adducts with α - and β -tubulin, which induce the aggregation of the microtubules required for the fusion of autophagy vacuoles and lysosomes.³⁹ In AD, toxic forms of tau

and A β affect the UPS system⁴⁰ and favour the formation of senile plaques and by paired helical filaments (PHFs) instead of degradation and clearance. In Down syndrome, A β accumulates with age in the frontal cortex and oxidative damage may contribute to the onset and progression of AD pathogenesis.⁴¹ In AD frontal cortex, several anti-oxidants such as glutathione, glutathione peroxidase, glutathione-S-transferase and superoxide dismutase are reduced and this suggests an oxidative stress-dependant reduction of oxidation defence mechanisms, contributing to mitochondrial dysfunction and loss of synapses.⁴²

Oxidation affects also directly the UPS, since several proteins of the proteasome are oxidized and also ubiquitinated, influencing protein repair and clearance. In addition, several proteins may have a toxic effect in vulnerable cells and induce cell death. One of these proteins is the Ubiquitin C-terminal hydrolase L1 (Uch-L1), which plays a central role in the UPS, and is essential for de-ubiquitination.⁴³ It was also shown to be required for normal synaptic and cognitive function, since it can restore A β -induced decrease of synaptic function and contextual memory in an AD mouse model.⁴⁴ It was found oxidized in AD brain and its soluble levels were inversely proportional to the number of neurofibrillary tangles (NFTs).^{30,45} These reports all point to an important role of the UPS in neurodegenerative diseases and suggest that protein alteration due to oxidation or to the lack of protein repair may strongly contribute to degenerative events.

Relationship between oxidation and ubiquitination

If we have to come up with a viable model for the generation of sporadic AD, it seems that age-related increase in oxidation and reduction of anti-oxidant defence mechanisms may play a major role in the generation of ROS via mitochondrial dysfunction in synapses, combined with a dysregulation of repair mechanisms. This may also depend on the individual defence mechanisms of cells and point to a selective cellular vulnerability for dysfunction of redox and ubiquitination mechanisms.

In Figure 1, we have summarized a variety of mechanisms that play an essential part in the formation of toxic products and in the lack of correct clearance of noxious byproducts eventually leading to cell death. Several proteins may play a key role and unless correctly cleared affect the cell metabolism. This is exemplified for tau proteins and for a particular A β peptide form, a derivative from amyloid precursor protein (APP). At first, oxidative damage resulting from oxidative stress at synaptic or cellular level may increase free radicals and protein oxidation. Such proteins may be repaired or eventually tagged for degradation by ubiquitination. During aging, this mechanism is disturbed^{46,47} and, instead of protein clearance, may result in the accumulation of ubiquitinated proteins in form of aggresomes.⁴⁸ Some proteins may even affect the correct function of the proteasome.⁴⁹ Depending on circumstances, vulnerable cells may respond to some of the modified proteins such as phospho-forms of tau proteins and/or micro-fibrils of tau as well as to specific cleavage forms of APP and

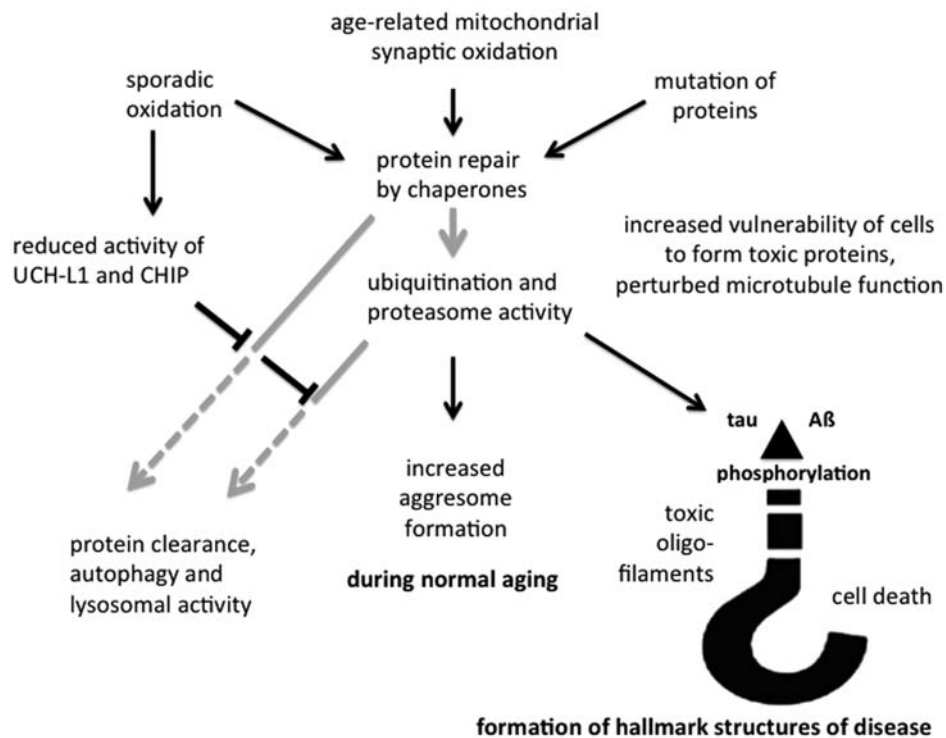


Figure 1 The normal pathway of protein repair, ubiquitination and protein clearance by the proteasome system is outlined with grey arrows. Different factors such as oxidative stress, mitochondrial deficits or mutations may lead to an increased oxidation of proteins. They may also affect mechanisms involved in protein repair and block protein clearance. As a consequence, proteins are becoming increasingly ubiquitinated and form aggresomes during normal aging. In addition, some of these proteins may even have a toxic effect in vulnerable neurons and lead to the formation of hallmark structures in neurodegenerative pathologies, and finally to cell death

consequently to A β forms and their toxic effects.^{16,50} However, recent results suggest that there is no direct correlation between the level of pathological tau and the extent of aggresome formation in different brain areas.⁵¹ Therefore, some neurons may be more vulnerable than others to the toxicity of some proteins in different parts of the brain.⁵² For example, we can mention the sporadic formation of a toxic triad of tau, A β and fyn in hippocampus and neocortex in AD,⁵⁰ the presence of abnormal phosphorylated neurofilaments in motoneurons in ALS,³⁸ the accumulation of huntingtin in the striatum in Huntington's disease⁵³ or the formation of toxic aminochromes in the substantia nigra in Parkinson's disease.³⁹

What are target mechanisms of potential interest?

A main objective in the search for therapeutic treatments is how to reduce protein toxicity and increase protein degradation and clearance.^{34,54} Recent attempts focus on the understanding of tau aggregation and how to interrupt the formation of toxic tau filaments.⁵⁵ Late onset AD provides a challenge to identify markers for different neurodegenerative diseases. There is a need for a clinical evaluation of several oxidation components in the blood such as protein carbonyls, nitrite and glutathione, catalase, α -tocopherol, calcium, Se and Zn as possible predictive markers of neurodegenerative diseases.^{56,57} Indeed, elevated labile Cu levels in cortical brain tissue were associated with oxidative stress in AD, as well as a deranged Cu homeostasis.⁵⁸ A causal role of mitochondrial bioenergetics deficits and brain hypometabolism coupled with increased mitochondrial oxidative stress is probable in AD, as well as a shift from glucose-driven to the lesser efficient ketogenic pathway.⁵⁹ Efficient strategies are based on compensation for oxidative stress related to synaptic mitochondria and favouring glucose-driven metabolic activities. A mitochondrion-specific anti-oxidant treatment for AD remains very hypothetical. However, using endogenous anti-oxidant defence mechanisms and an appropriate nutrition plan of anti-oxidant ingredients such as citrulline, taurine, creatine, polyphenols, selenium, zinc, vitamin A, C and E may help to reduce progression of mental decline.^{27,29,52} Targeting factors that damage mitochondria with acetyl-L-carnitine or R-alpha-lipoic acid helps reversing effects of mitochondrial damage and eliminating the imbalance in energy production and A β oxidation, thus making antioxidants a promising alternative for an early AD prevention.²⁸ Xanthene food dye, such as erythrosine B, was reported as novel modulator of A β aggregation and reduced A β -associated impaired cell function and becomes an attractive molecule since it is already Food and Drug Administration (FDA)-approved.⁶⁰ N-acetyl-cysteine is another promising molecule to improve action of glutathione as therapeutic strategy to reduce oxidative stress⁶¹, because it can reverse memory impairment in aged SAMP8 mice⁶² or reduce oxidative stress in Schizophrenia.⁶³ However, to test the efficacy of any treatment or specific diets, it is essential to have specific tools to measure and quantify oxidation, S-nitrosylation or carbonylation levels in blood and cerebrospinal

fluid and to determine target proteins involved in oxidative stress.^{30,64}

Conclusion

In neurodegenerative diseases, a variety of cellular mechanisms are involved and are in relation to ongoing basic cellular events. The initial question, whether oxidation and ubiquitination is essential, can be answered with a clear **Yes**. Oxidative stress affects many processes in mitochondria and synapses with consequences for repair mechanisms and aggresome formation as well as pathological structures in a variety of neurodegenerative diseases. However, oxidation and UPS dysregulation is probably not the main cause for most neurodegenerative diseases, for the exception of ALS where SOD1 mutations are detrimental. Protein oxidation and conformational changes may contribute to changes in protein function and so influence the progression of neurodegeneration. One point that transpires is that oxidation of proteins is a non-negligible factor during aging and may provide many targets to develop therapeutic strategies how to improve the redox balance. Furthermore, repair mechanisms are essential to get the specific pathways on the right track and correct errors, to grant a proper metabolic control and to favour correct protein repair.

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