A REVIEW ON THE CAUSE-EFFECT RELATIONSHIP BETWEEN OXIDATIVE STRESS AND TOXIC PROTEINS IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

Liana Rada Borza
University of Medicine and Pharmacy “Grigore T.Popa” – Iași
Faculty of Medicine
Clinical Hospital of Psychiatry “Socola” – Iași

A REVIEW ON THE CAUSE-EFFECT RELATIONSHIP BETWEEN OXIDATIVE STRESS AND TOXIC PROTEINS IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES (Abstract): Protein aggregates are the defining pathological feature of human neurodegenerative diseases. Studies have revealed that mutant huntingtin, polyglutamine-expanded ataxin-1 and ataxin-3 can cause elevated levels of reactive oxygen species in neuronal cells. It has also been indicated that the normal host prion protein behaves as an antioxidant, while the neurotoxic peptide based on the sequence of the scrapie isoform increases hydrogen peroxide toxicity in neuronal cultures. Additionally, not only can oxidative stress contribute to the aggregation of β-amyloid and α-synuclein, but both β-amyloid and α-synuclein can induce oxidative damage. Furthermore, oxidative stressors have been shown to play a critical role in neurofibrillary pathology leading to tau hyperphosphorylation. In conclusion, the present review supports a cause-effect relationship between oxidative stress and toxic proteins in the pathogenesis of neurodegenerative disorders. Keywords: NEURO-DEGENERATIVE DISEASES, PROTEIN MISFOLDING, OXIDATIVE STRESS

Neurodegenerative disorders as diverse as Parkinson’s disease, prion diseases, polyglutamine diseases, Alzheimer’s disease and related tauopathies share a common feature – aggregation and deposition of abnormal protein. Expression of mutant proteins in transgenic animal models recapitulates features of these diseases. Neurons are particularly vulnerable to the toxic effects of mutant or misfolded proteins. When they accumulate in sufficient quantity, misfolded proteins are prone to aggregation. Failure to detect and eliminate misfolded proteins contributes to the pathogenesis of neurodegenerative disease (1).

As previously mentioned, the common mechanistic theme of these diseases is the formation of aggregates containing misfolded proteins with a concomitant gain of function which eventually leads to neuronal death. A gain of function, rather than a loss of function, is sustained by the observation that the aggregates are toxic per se. Several studies have addressed the following question: what makes the aggregates toxic? In this regard, a widely invoked cause of membrane damage has been free radical oxidative stress (2). The oxidative damage induced by the redox activity of a target protein, which interacts with free radicals and metal ions, has been found as a typical hallmark in the majority of neurodegenerative
disorders. Thus, protein aggregation and oxidative stress have been demonstrated to be the major factors involved in the neurodegenerative process (3).

The objective of the current review would be to examine a possible cause-effect relationship between oxidative stress and the toxic proteins in the pathogenesis of neurodegenerative diseases.

POLYGLUTAMINE DISEASES

**Huntington's disease (HD)** is an autosomal dominant neurodegenerative disease caused by an expansion of cytosine-adenine-guanine (CAG) repeats located in the coding region of the IT15 – interesting transcript 15 – gene on chromosome 4, whose product is named huntingtin (htt) (4). In addition, intranuclear inclusion bodies have been found in the striatal neurons of transgenic mice expressing the CAG repeat containing exon 1 of the htt gene (5). The expanded polyglutamine (polyQ) tract contained by the encoded protein htt may be toxic directly and/or through interactions with other cellular proteins (6).

The interaction between the mutated N-terminal part of htt and the glycolysis enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) supports a role for oxidative damage in the disease process, as it impairs the glycolitic activity of this enzyme. The use of spectrophotometric assays in postmortem brain tissue has revealed impaired oxidative phosphorylation enzyme activity restricted to the basal ganglia in HD brain (7). GAPDH interaction with htt has been further increased by the presence of the expanded polyQ stretch (8).

It has been observed that mutant htt (mhtt) causes increased levels of reactive oxygen species (ROS) in neuronal SK-N-SH (human neuroblastoma) cells and non-neuronal COS-7 (monkey kidney) cells. Thus, it has been postulated that endogenous heat shock protein 27 (HSP27) in its unphosphorylated form suppresses polyQ-mediated cell death by protecting against ROS induced by mhtt. It has been shown that HSP27 mutants showing protection in the polyQ model increase survival of HeLa cells exposed to hydrogen peroxide (H₂O₂). Oxidative stress is likely to be relevant to polyQ-induced death, since both N-acetyl-L-cysteine and the reduced form of glutathione (GSH) have inhibited polyQ-mediated cell death. Furthermore, cells expressing mhtt have presented decreased levels of GSH, which have been increased by co-expression of HSP27 (9).

A consequence of the defect in energy metabolism caused by mhtt may be an increase in oxidative injury, a regionally specific mechanism of neuronal death in HD. A well-established marker of oxidative damage to deoxyribonucleic acid (DNA) is 8-hydroxy-2-deoxyguanosine (OH8dG), and a significant increase in OH8dG in nuclear DNA has been found in the caudate nucleus in HD post-mortem tissue (7). A significant increase in OH8dG has also been found in mitochondrial DNA of parietal cortex from HD patients (10).

In addition, lipid peroxidation (LP), induced by the formation of free radicals, has been determined in the striatum, cortex and cerebellum of R6/1 transgenic mice that express a human mutated htt on transgenic mice that express a human mutated htt with approximately 116 CAG repeats, and wild-type (WT) control mice at 11, 19, 24 and 35 weeks of age. A tendency of elevated levels of LP in the R6/1 mice has started by 19 weeks and has become significant by 24 weeks; at 35 weeks, LP in transgenic striatum was 274% higher than in non-transgenic mice. As a result, mutated htt specifically...
induces progressive oxidative damage to the striatum of transgenic mice (11).

**Spinocerebellar ataxia 1 (SCA1)** is an autosomal dominant neurodegenerative disorder caused by the expansion of CAG trinucleotide repeats encoding the polyQ tract in ataxin-1, the SCA1 gene product. PolyQ expansion leads to the aggregation of ataxin-1 proteins in the nucleus. It has been shown that the large aggregates formed by polyQ-expanded ataxin-1 recruit the Cu/Zn-binding isoform of superoxide dismutase, SOD1, in the nucleus of HeLa cells. In the case of mutant ataxin-1-expressing cells, most of SOD1 has been detected in nuclear aggregates; in the case of normal ataxin-1-expressing cells, some Cu/Zn-SOD has been found co-localized in ataxin-1 aggregates and some diffused in the cytosol. In addition, it has been revealed that the oxidation of intracellular proteins occurs with a higher frequency in the presence of mutant ataxin-1 relative to normal ataxin-1. When treated with both BSO (an inhibitor of γ-glutamylcysteinyl synthetase, the first enzyme in the GSH biosynthetic pathway) and t-butyl hydroperoxide (TBH), mutant ataxin-1-expressing cells have proved to be more prone to mitochondrial dysfunction than normal ataxin-1-expressing cells. These results suggest that polyQ-expanded ataxin-1-expressing cells increase the levels of ROS in HeLa cells (12).

**Spinocerebellar Ataxia 3 (SCA3)/Machado-Joseph Disease.** Human SK-N-SH neuroblastoma cells stably transfected with full-length mutant Machado-Joseph disease (MJD) with 78 CAG repeats have been established to analyze the role of mutant ataxin-3 and mimic an early disease stage, as less than 1% of the mutant cells contain nuclear aggregates under basal conditions. To test the hypothesis that oxidative stress might contribute to the progression of the disease, TBH has been used to assess the oxidative tolerance of cells. It has been noticed that SK-N-SH-MJD78 cells, stably expressing expanded ataxin-3, are more susceptible to low concentrations of TBH (1 and 3 μM) up to 24h treatment than the parental SK-N-SH cells. Additionally, Western blot analysis has indicated that the protein expression of HSP27 has dramatically decreased in both neuronal and non-neuronal cells with expanded ataxin-3, which is possible to result in an increase of ROS in the cellular model. The same significant reduction of HSP27 has been further confirmed in the lymphoblastoid cell lines from two MJD patients, compared with that from a normal individual. Semiquantitative reverse transcription-polymerase chain reaction and microarray analysis have ruled out the possibility that the reduced HSP27 expression could be due to transcriptional repression of HSP27 gene, further supporting the evidence that expanded ataxin-3 increases the vulnerability to oxidative stress (13).

**PRION DISEASES**

According to “prion hypothesis” formulated by Prusiner in 1982, transmissible spongiform encephalopathies are caused by “novel proteinaceous infectious particles” corresponding to the scrapie isoform (PrPsc) of the normal host prion protein (14).

It has been suggested that the conformational conversion of normal, protease-sensitive, primarily α-helical form of prion protein (the cellular isoform, PrPc) into a pathogenic, protease-resistant, β-sheet-rich form of the protein (PrPsc) may be the result of imbalances in the level of copper. The changes in copper concentration have
altered prion protein (PrP) ability to act as an antioxidant by modifying its conformation. Truncated PrP could be a result of self-proteolysis by PrP when Cu²⁺ physiological concentration is too high (15). Moreover, it has been reported that PrPc undergoes a site-specific cleavage, both Cu²⁺- and ph-dependent, of the octapeptide-repeat region on exposure to ROS such as H₂O₂ and superoxide ions (O₂⁻). Such ROS cleavage might be involved in the formation of the scrapie isoform, as abnormal cleavage of PrPsc occurs into the octarepeat region (16).

The flexible amino-terminus of PrPc contains the octapeptide PHGGGWGQ, which is repeated four times and is among the best-preserved regions of mammalian PrPc. Proton nuclear magnetic resonance (NMR) spectroscopy has indicated that the histidine residues in each octarepeat are coordinated to the copper ion. It has been concluded that host PrPc gains a Cu,Zn-SOD-like antioxidant activity, dependent on copper incorporation (17). Immunoprecipitated PrPc from WT mouse brain has also mimicked SOD1 activity, which confirms that native PrP behaves as an antioxidant. Additionally, PrPc dependent SOD1 activity has been abolished by the precise deletion of the octapeptide-repeat region (18). Moreover, native PrPc, affinity-purified from mouse brain, has exhibited the same SOD1-like antioxidant activity, as determined by spectrophotometric assay. In conjunction, incubation of purified PrPc with PrP106-126, the neurotoxic peptide based on the sequence of PrPsc, has significantly inactivated the SOD1-like activity (19).

It has also been demonstrated that SOD1 activity varies with the level of PrPc expression. Thus, prion protein-deficient cells proved to be more sensitive to oxidative stress due to reduced SOD1 activity (20). In addition, SOD1 activity has proved to be significantly higher in cerebellar cells overexpressing PrP than for WT extracts. Moreover, the expression of PrPc may regulate Cu, Zn-SOD activity by influencing copper incorporation into the SOD1 molecule (21).

Furthermore, a study on 8 week-old prion protein knock-out mice (Prnp/-) has emphasized a role for PrP in modulating oxidative homeostasis in vivo. In this regard, higher levels of oxidative damage to proteins and lipids have been found in the brain lysates of Prnp/- as compared to WT mice of the same genetic background (22). PrP/- neurons also proved to be significantly more susceptible to H₂O₂ toxicity than WT neurons after a 6 and 24 hour exposure. This result might be related to the significant decrease in glutathione reductase (GR), but not glutathione peroxidase (GPx), activity detected in PrP/- neurons both in vitro and in vivo. These findings have been supported by PrP106-126 that has significantly inhibited GR activity and increased H₂O₂ toxicity in neuronal cultures (23).

ALZHEIMER’S DISEASE & PICK’S DISEASE
The presence of intracellular neurofibrillary tangles, containing hyperphosphorylated tau protein and apolipoprotein E, and of extracellular senile (neuritic) plaques, including non-soluble β-amyloid, ubiquitin, apoE, presenilins and alpha-antichymotrypsin, are considered hallmarks of Alzheimer’s disease (AD) (24). AD is associated with an increased cerebral accumulation of amyloid beta-protein (Aβ), a 39-42 amino acid peptide. Soluble Aβ monomers aggregate to form antiparallel β-
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Pleated sheets as proved by circular dichroism studies (25).

As Aβ is considered to have a causal role in AD, the effect of oxidative stress on its metabolism within the cell has been studied. It has been reported that oxidative stress induced by H₂O₂ promotes intracellular accumulation of Aβ in human neuroblastoma cells through enhancing the amyloidogenic pathway (26). A possible role for LP in the pathogenesis of AD has also been suggested. It has thus been determined that membranes containing oxidatively damaged phospholipids promote beta-sheet formation in a 42-residue Aβ and accumulate Aβ significantly faster than membranes containing normal saturated phospholipids (27).

It has also been shown that H₂O₂ mediates Aβ-induced neurotoxicity, which explains why catalase, that degrades H₂O₂, protects the cells from β-amyloid toxicity. In addition, Aβ has induced the activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa B), a transcription factor thought to be regulated by oxidative stress (28). It has been suggested that amyloid peptides including Aβ may be toxic via a common oxidative mechanism and that their amphiphilic form, rather than the structure in β layers, may be the cause (29, 30).

Moreover, it has been emphasized that, under some conditions, toxic Abeta peptides Aβ1-40 and Aβ25-35 enhance transition metal-catalyzed oxidation of hydroxylamine derivatives (31). Several studies have also concluded that cortical or hippocampal synaptosomes isolated from AD brain or from rodent brain or rodent cultured hippocampal neurons incubated with Aβ1-42, Aβ1-40 or Aβ25-35 have increased protein oxidation; non-toxic reverse peptides have not caused protein oxidation (32).

It can thus be proposed the amyloid β-peptide-associated free radical oxidative stress model for neurotoxicity in AD: Aβ-initiated ROS react rapidly with several moieties in the plasma membrane and cause protein oxidation and LP; toxic products of LP, such as 4-hydroxy-2-nonenal and acrolein (2-propenal), having longer half-lives than free radicals, migrate to different parts of the neurons causing multiple alterations of cellular function such as sharp increases in intracellular Ca²⁺ and ultimately leading to neuronal death. Moreover, it has been suggested that methionine-35 (Met-35) is a key amino acid involved in Aβ radicalization, aggregation and neurotoxicity, since it is the most susceptible Aβ residue to oxidation in vivo, especially under oxidative stress conditions. Methionine oxidation to the sulfoxide leads to predominantly β-sheet conformation adopted by toxic Aβ. Examination of senile plaque-resident Aβ1-40 has detected a high proportion of methionine sulfoxide (33, 34).

However, it has been indicated that, rather than being a source of ROS, β-amyloid may be a modulator of ROS production (35).

Furthermore, the hyperphosphorylation of the microtubule-associated protein tau (τ) is considered a hallmark of tauopathies such as AD and Pick’s disease. It has been hypothesized that oxidative stressors play a critical role in neurofibrillary pathology by activating stress-activated protein kinases such as p38 pathway that leads to τ phosphorylation in vitro and co-localizes with phosphorylated τ in vivo (36). It has been found that acrolein, a LP product from arachidonic acid, increases τ phosphorylation due to p38 stress-activated kinase (37).
PARKINSON’S DISEASE & DEMENTIA WITH LEWY BODIES

The synucleins (α, β and γ-synuclein) are a small family of proteins highly expressed in nervous tissue. An obvious link with Parkinson’s disease (PD) has been established by showing that two missense mutations in the α-synuclein gene (A53T and A30P) are responsible for rare familial forms of the disease (38). In both PD and dementia with Lewy bodies α-synuclein accumulates in the form of fibrillar aggregates inside the Lewy bodies, cytoplasmic inclusions deposited within dopaminergic neurons.

Alpha-synuclein appears to increase ROS levels in dopaminergic neurons via more than one process. First, α-synuclein interacts with the dopamine (DA) transporter and facilitates its clustering at the plasma membrane. Consequently, DA uptake is accelerated leading to increased susceptibility to DA-induced apoptosis. Therefore, the re-uptake of more DA intracellularly can be a source of increased ROS due to DA metabolism (39). It has been indicated that over-expression of α-synuclein and especially its mutant forms exaggerates the vulnerability of neurons to DA-induced cell death through excess intracellular ROS generation (40). Additionally, transfection of cell lines with mutant forms of α-synuclein have increased the baseline levels of oxidative damage, reflected by decreased levels of GSH and enhanced levels of 8-hydroxyguanosine (8-OHG), protein carbonyls and LP (41).

It has also been shown that both full-length α-synuclein and its synthetic peptide fragment, when incubated in solution, have the ability to generate hydroxyl radicals upon the addition of ferrous ion (42). In conjunction, it has been revealed that oxidative stress can induce the aggregation of human α-synuclein (43). This bi-directional molecular pathway could create a vicious cycle of α-synuclein changes and excessive oxidative stress.

Moreover, it has been shown that parkin, an ubiquitin-protein ligase, interacts with α-synuclein; double staining of PD brains has illustrated that parkin and α-synuclein co-localize to the same pathological structures (both Lewy bodies and axonal spheroids) (44). In at least one patient, mutations in parkin have led to Lewy body formation as seen in sporadic PD. It has been investigated how overexpression of WT and mutant parkin proteins modulates proteasomal activity, ubiquitinated proteins accumulation, oxidative stress indices or antioxidant defenses. Increasing expression of WT parkin by gene transfection in two cell lines has led to increased proteasomal activity, decreased levels of protein carbonyls and a trend to a reduction in ubiquitinated protein levels. Transfection of the two cell lines with DNA encoding three mutant parkins associated with autosomal recessive juvenile parkinsonism have not altered antioxidant enzyme activities, namely SOD1, SOD2, catalase, GPx and GR, but have increased oxidative stress, as indicated by decreased levels of GSH, smaller increases in proteasomal activity and elevated levels of protein carbonyls and LP (45). Impairment of proteasomal function leads to free radical generation and oxidative stress (46). Therefore, altered activity of the ubiquitin-proteasome system, which degrades oxidatively damaged proteins, may play a key role in PD pathogenesis.

CONCLUSIONS

The present review provides strong evidence for a cause-effect relationship be-
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between oxidative stress and toxic proteins in the pathogenesis of neurodegenerative diseases.

In this regard, it has been suggested that a toxic gain of function of mutant htt arising from altered interactions with other proteins may lead to oxidative damage. In addition, transgenic mice over-expressing the mutated amino terminus of human htt have been shown to suffer the effects of increased oxidative damage. Moreover, studies have indicated that both polyglutamine-expanded ataxin-1 and ataxin-3 increase the vulnerability to oxidative stress.

It has also been emphasized that the loss of resistance to oxidative stress due to inactivation of the function of the normal form of prion protein, either due to conversion or through interaction with the pathogenic form of the protein (the scrapie isoform), may be important to the pathogenesis of prion diseases.

Furthermore, several studies have demonstrated not only that the oxidative processes can contribute to the aggregation of β-amyloid, but that β-amyloid itself can be considered a source of free radicals, thus supporting amyloid β-peptide-associated free radical oxidative stress model for neurotoxicity in AD.

Finally, this review points out that α-synuclein increases ROS levels in dopaminergic neurons and oxidative stress can induce the aggregation of α-synuclein.

REFERENCES

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THE INFLUENCE OF BAROREFLEX ACTIVATION THERAPY ON RENAL RESPONSES IN PATIENT WITH RESISTANT HYPERTENSION

It is widely accepted that renal system play an essential role in terms of long-term blood pressure regulation. Recently, a non-pharmacological therapy represented by baroreflex activation has provided to be a promising alternative in order to reduce arterial pressure, particularly in patient with drug-resistant hypertension. A multicentre, double blind Rheos Pivotal Trial was designed to investigate the renal response and efficiency to prolonged baroreflex activation therapy in patient with drug resistant hypertension. 322 patients aged 21 to 80 years, who had blood pressure ≥ 160/80 mmHg despite maximal tolerated therapy with ≥ 3 antihypertensive medication including a diuretic, were divided in two groups. Group 1 consisted of 236 patients who started baroreflex activation therapy 1 month after device implantation, whereas in the 86 patients from group 2 the device was activated 6 months later (deferred baroreflex activation therapy). Serum creatinine, urine albumin/creatinine ratio and estimated glomerular filtration rate were determined to assess renal function. The results showed that in group 1, serum creatinine increased from 78 to 84 μmol/L, and glomerular filtration rate decreased from 92 to 87 mL/min per 1.73m² at month 6, whereas albumin/creatinine ratio did not change. Group 2 demonstrated similar renal responses at month 6 and month 12 as group 1. In conclusion, chronic baroreflex activation does not result in further decrease in renal function, suggesting that this non-pharmacological approach is safe, effective and promising. (Alnima T, De Leeuw PW, Tan F, Kroon A. Renal Responses to Long-Term Carotid Baroreflex Activation Therapy in Patients With Drug-Resistant Hypertension. Hypertension 2013; 61(6):1334-9). Ionut Tudorancea