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REVIEW ARTICLE

Oxidative and Inflammatory Events in Prion Diseases: Can they Be Therapeutic Targets?

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Abstract: Prion diseases are a group of incurable infectious terminal neurodegenerative diseases caused by the aggregated misfolded PrPsc in selected mammals including humans. The complex physical interaction between normal prion protein PrPc and infectious PrPsc causes conformational change from the α- helix structure of PrPc to the β-sheet structure of PrPsc, and this process is repeated. Increased oxidative stress is one of the factors that facilitate the conversion of PrPc to PrPsc. This overview presents evidence to show that increased oxidative stress and inflammation are involved in the progression of this disease. Evidence is given for the participation of redox-sensitive metals Cu and Fe with PrPsc inducing oxidative stress by disturbing the homeostasis of these metals. The fact that some antioxidants block the toxicity of misfolded PrPc peptide supports the role of oxidative stress in prion disease. After exogenous infection in mice, PrPsc enters the follicular dendritic cells where PrPsc replicates before neuroinvasion where they continue to replicate and cause inflammation leading to neurodegeneration. Therefore, reducing levels of oxidative stress and inflammation may decrease the rate of the progression of this disease. It may be an important order to reduce oxidative stress and inflammation at the same time. This may be achieved by increasing the levels of antioxidant enzymes by activating the Nrf2 pathway together with simultaneous administration of dietary and endogenous antioxidants. It is proposed that a mixture of micronutrients could enable these concurrent events thereby reducing the progression of human prion disease.

Keywords: Oxidative stress, inflammation, apoptosis, antioxidants, misfolded proteins, spongiform encephalopathy, prion diseases.

1. INTRODUCTION

Prion diseases are a group of rare progressive incurable transmissible infectious neurodegenerative diseases caused by aggregated misfolded β-sheet of PrPsc protein in selected mammals including humans. As early as 1730's, the symptoms of prion disease were known as scrapie in sheep and goat. In 1957, a transmissible neurological disease called kuru, similar to Creutzfeldt-Jakob disease (CJD), was identified in the Fore tribe of Papua, New Guinea [1]. It was found that extracts from the autopsied brain of individuals with kuru when administered into chimpanzees led to similar brain pathology [2]. A similar cross species infectivity attributed to beef consumption was found in the United Kingdom following an outbreak of "mad cow disease". This is known as variant CJD (vCJD). In 1982, Dr. Stanley Prusiner of the University of California School of Medicine, San Francisco, proposed the term prion. He isolated an infective agent scrapie that induced neurodegeneration in the brain of sheep and goats [3]. A similar infectious agent was isolated from the brains of victims of the genetic diseases CJD and Gerstmann–Sträussler–Scheinker syndrome (GSS). In 2012, it was suggested that neurodegenerative diseases such as Alzheimer's Disease (AD) could be considered a prion disease [4]. Thus beta-sheet Aβ peptides of AD are not infectious and do not cause Transmissible Spongiform Encephalopathy (TSE), whereas the β-sheet of PrPsc is infectious and causes TSE.

Several studies have found that increased oxidative stress [5, 6] and inflammation [7-9] are associated with the progression of prion disease. In addition, an interaction between redox-sensitive metals [primarily copper (Cu) and iron (Fe)] and PrPc by altering the homeostasis of these metals may contribute to increased oxidative stress [10, 11]. The involvement of oxidative stress in the pathogenesis of prion disease is further supported indirectly by reports that antioxidants can reduce neurotoxicity both in cell culture and in animal models [12-15]. Therefore, reducing oxidative stress and inflammation appears to be a rational choice for slowing down the progression of prion diseases.

This review briefly describes incidence, forms, transmission, symptoms, and pathology of prion disease, and discusses factors facilitating the transition of normal prion pro-

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tein (PrPc) to infectious prion protein (PrPsc), modes of transport and translocation of PrPsc from the periphery to the brain. Evidence showing that increased oxidative stress and inflammation are involved in the progression of this disease is assembled. This includes support for the interaction of redox-sensitive metals Cu and Fe with PrPc and PrPsc leading to disruption of normal intracellular homeostasis of the metals, resulting in increased oxidative stress. This review proposes that in order to reduce oxidative stress and inflammation at the same time, it is critical to increase the levels of antioxidant enzymes by activating the Nrf2 pathway, at the same time as supplementation with dietary antioxidant compounds. The use of a mixture of micronutrients that would bring these changes about is advocated as a possible means of reducing the rate of progression of human prion disease.

2. INCIDENCE, FORMS, AND TRANSMISSION OF PRION DISEASES

The annual incidence of prion disease Creutzfelt-Jacob Disease (CJD) in the USA is about 1-case/million persons [16]. Human prion diseases include sporadic CJD (sCJD), the most common form (about 85% of cases) which involves a spontaneous mutation, and less-common inherited forms included, familial CJD (fCJD), Fatal Familial Insominia (FFI), and Gerstmann–Sträussler–Scheinker syndrome (GSS). Animal prion diseases include Bovine Spongiform Encephalopathy (BSE), scrapie of sheep, and chronic wasting disease of deer and elk. These can be transmitted to humans by consumption of infected meat. Sporadic CJD results from spontaneous conversion of PrPc to PrPsc in some carriers of a specific mutation that enhances this possiblitiy, rather than from PrPsc infection from external sources [17, 18]. This transition which does not involve genetic changes may take place spontaneously especially with some variants of PrPc. Familial Creuztfeldt-Jacob Disease (fCJD) is found in a small population of Libyan Jews that have extensively interbred for centuries. The clinical and pathological features of fCJD in this community are similar to those observed in sCJD, but the incidence of this disease in this community is 100 times higher than in general population [19]. The fCJD in this community is linked to the E200K mutation (substitution of glutamate for lysine at codon 200) in PRNP gene. Another fCJD linked to the mutation in which a substitution of Valine (V) for Glycine (G) at codon 114 (G114V) is found in a single Chinese patient [8]. Iatrogenic CJD occurs when the infectious agent is transmitted from person to person by medical/surgical procedures such as blood transfusion, and contaminated dental tools [20, 21]. The progression of various types of prion disease can vary in its rapidity, area of brain primarily affected and the nature of its clinical progression.

3. SYMPTOMS OF PRION DISEASE

sCJD is characterized by rapid progressive dementia. Earlier symptoms include muscular incoordination, impaired memory, judgment, thinking, and vision. Individuals with CJD may suffer from insomnia, depression, or unusual sensation. Pneumonia and other infections often precipitate death. The symptoms of vCJD are characterized by a longer period incubation period and by the relatively early onset of psychiatric symptoms, (CDC, 2017). These may include social isolation, delusional ideation, irritability/aggression, visual hallucinations, anxiety, and depression. Sporadic CJD occurs mostly in older individuals with rapid progression of dementia leading to death within a year, whereas variant vCJD is found in younger individuals with slower progression of the cognitive dysfunction [22, 23].

4. PATHOLOGY OF PRION DISEASES

The pathological changes in the brain of CJD patients are also complex, depending upon the type of mutation in PRPN gene, regions of the brain, and type of PrPsc. The extent of vacuolation (spongiform change) and deposition of PrPsc differ in various regions of the brain. In the case of sCJD, the density of vacuolation is highest in the occipital cortex and cerebellum and lowest in the dentate gyrus, whereas the degree of deposition of PrPsc is similar in the cortex and cerebellum, but they were absent in the dentate gyrus [24]. The clinical changes in sCJD patients consist of rapid progressive cognitive dysfunction, diffusion-weighted magnetic resonance imaging (DWI) hyperintensity, myoclonus, periodic sharp-wave complexes on electroencephalogram, and akinetic mutism state. Pathological alterations in the brain included spongiform changes in the gray matter, gliosis, and neuropil rarefaction, followed by neuronal loss. Changes in the levels of spongiform occur several months before gliosis and the emergence of symptoms. [25].

5. TRANSLOCATION OF EXOGENOUSLY INFECTED PrPsc FROM THE PERIPHERAL TISSUES TO THE BRAIN

The mechanisms of translocation of exogenously infected PrPsc from the peripheral tissue to the brain in humans are not well understood. From studies in mice, it appears that mononuclear phagocytes play an important role in translocation processes in prion disease. Some phagocytes may help PrPsc entry into lymphoid tissues where they propagate, whereas others may remove PrPsc by phagocytosis. The same study reported that an intact splenic marginal zone permitted the rapid delivery of PrPsc into B-lymphocyte follicles where they replicated on the follicular dendritic cells prior to translocation to, and infection of the brain [26]. Other studies suggested that exogenously infected PrPsc enter lymphoid organs where they can replicate in the presence [27] or in the absence [28] of follicular dendritic cells prior to invasion of the brain.

6. TRANSITION OF PrPc TO PrPsc AND MECHANISMS OF PROLIFERATION OF PRPsc

Mutations in PRNP gene coding for PrPc can trigger conformational change of the normal α-helix structure of PrPc to the abnormal β-sheet structure of PrPsc. These form intracellular aggregates not susceptible to proteolytic degradation which can lead to neurodegenerative changes. PrPsc is infectious and in this, resembles bacteria and viruses [29]. However, unlike bacteria and viruses, PrPsc contains no nucleic acids [30, 31]. The mechanisms of transition from PrPc to PrPsc structure and subsequent replication of the PrPsc configuration is likely to involve a complex physical interaction between PrPc and PrPsc at the cell surface. The newly

formed PrPsc structures can accumulate as intracellular aggregates or at the cell surface [29]. This transition from PrPc to PrPsc repeated many times, leads to a chain reaction and an exponential increase in the number of PrPsc particles [32]. In the familial variant of prion disease, spontaneous generation of PrPsc may be due to selective migration of mutant PrPc to the acidic environment of the lysosome that facilitates the conversion of PrPc to PrPsc [33]. A substantial degree of conversion of PrPc to PrPsc is likely to occur in the endomal/lysosomal system in all prionoses.

7. POLYMORPHISMS OF THE PrPc GENE

Polymorphisms in the PrPc gene strongly influence susceptibility of prion disease [34]. The PrPc allele PrP^{VRQ} is present in a significant number in scrapie-infected cells, whereas the other allele PrP^{ARR} is found only in healthy cells. Two other alleles PrP^{ARQ} and PrP^{ARH} are present in both infected and uninfected sheep cells in similar number. Rov cells (derived from RK13 cell line of normal rabbit kidney epithelial cells) expressing an ovine PrPc allele PRP^{VRQ} are very sensitive to sheep prion transmission and replication, whereas Rov cells expressing Prpc allele PrP^{ARR} are resistant to prion infection [35].

Polymorphism of the human PRNP gene, methionine (M)/valine (V) at codon129 and glutamic acid (E)/lysine (K) at codon 219 affect the sensitivity of host to prion disease. 129M/M homozygotes are overexpressed in patients with sCJD and vCJD, while 219E/K heterozygotes are absent in sCJD [36]. Although 219E/K confers resistance against the development of sCJD, this genotype does not confer the same protection in acquired forms (iatrogenic CJD and vCJD) or genetic forms (genetic CJD and GSS) of prion disease [36]. A mutation at codon 178 (Asp178/Aspn) is associated with FFI and fCJD disease, depending upon the presence of Met or Val at codon129 respectively. Polymorphic forms of D178N human prion protein can exhibit enhanced rat of conversion from PrPc to PrPsc at acidic pH, and to thioflavin T-positive amyloid fibrils at neutral pH. A high rate of conversion to PrPsc is dependent upon the M/V polymorphism at 129. No such high rate of conversion is evident in the wild-type protein [37].

8. INTERACTIONS OF REDOX-LABILE METALS COPPER (Cu) AND IRON (Fe) WITH PrPc AND PrPsc

The interactions between redox-labile metals and PrPc and PrPsc in causing neurodegeneration are very complex. PrPc binds with redox-labile metals Cu and Fe and can act as a scavanger of free radicals [38]. However, these metals can lead to aggregation of PrPc [38, 39]. On the other hand, interaction between these redox-sensitive metals and the abnormal PrPsc leads to increased oxidative stress that is a factor contributing to neurodegeneration in prion diseases [10]. *In vitro* studies suggest that PrPc facilitates normal uptake and metabolism of copper and iron, while PrPsc may induce imbalance in metal homeostasis in prion disease [40-42]. In an isolated system Cu^{2+} induced misfolding of normal PrPc monomers and these misfolded monomers had a much higher affinity for copper than the original native isoform of this monomer, and this promoted their oligomerization [11]. PrPc-derived copper-binding peptide fragments in the helical region catalyze the production of superoxide anion in the presence of monoamines which can promote oxidative stress [43]. Cu and Fe may remain associated with PrPsc, and thereby, making the complex continually redox active thereby furthering neurodegeneration [39]. Redox active metal-induced oxidative stress caused aggregation of PrPc that is toxic in cell culture [39]. In contrast, these metals also promoted degradation of PrPsc by hydroxylation and thus decreased its infectivity [44]. Thus increased oxidative stress can enhance production of toxic PrPsc as well as leading to its degradation.

9. TRANSPORT OF PrPsc FROM INFECTED TO UNINFECTED TISSUES

Exosomes, membranous vesicles secreted into the extracellular spaces, may serve as shuttles for the transport of PrPsc from infected to uninfected tissues. The ceramide and Endosomal Sorting Complex Required for Transport (ESCRT-0) plays an important role in the biogenesis of exosomes; and may also play a role in the formation, release, and spread of PrPsc. Silencing HRS-ESCRT-0, a subunit hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) of (ESCRT-0), markedly reduces the adoption of a PrPsc configuration. Depletion of ESCRT-1 complex subunit tsg101 or reduction in the levels of ceramide significantly decrease the release and migration of PrPsc [45, 46]. These results suggest that ESCRT-dependent pathways are important in the release of PrPsc. Increased levels of PrPsc induce Endoplasmic Reticulum (ER) stress and lead to an activated Unfolded Protein Response (UPR). A major chaperone protein of the ER, GRP78/Bip, decreases ER stress levels and reduces apoptosis. Reduction in levels of GRP78 accelerates the progression of prion disease [47]. In the acquired prion disease, infection is initially propagated in the lymphoid tissue before invading and spreading in the brain. Cell free media derived from culture of infected neuronal cells contains PrPsc within exosomes, and these can transport PrPsc to uninfected cells [48]. After infection with sheep PrPsc, both PrPc and PrPsc are released into the extracellular environment where they are associated with exosomes [49]. Plasminogen markedly stimulates propagation of the PrPsc format in a dose-dependent manner by increasing the rate of generation of this transmissible agent [50].

The sequence of steps whereby PrPc is converted to (PrPsc), and migration of PrPsc to the brain, eventually leading to spongiform encephalopathy and death, is summarized in Fig. (**1**).

10. ROLE OF OXIDATIVE STRESS IN PRION DISEASE

Increased oxidative stress plays a central role in the initiation and progression of several neurodegenerative diseases which include familial, sporadic, and infectious forms of prion disease. Several studies suggest that oxidative events may be one of the important factors in conversion of normal prion protein (PrPc) to misfolded infective protein PrPsc.

More than 30 mutations in PRNP gene coding for PrPc protein are associated with familial prion disease [5] of which E200K-associated familial CJD is the most common [51]. Increased lipid peroxidation is one of the earliest signs

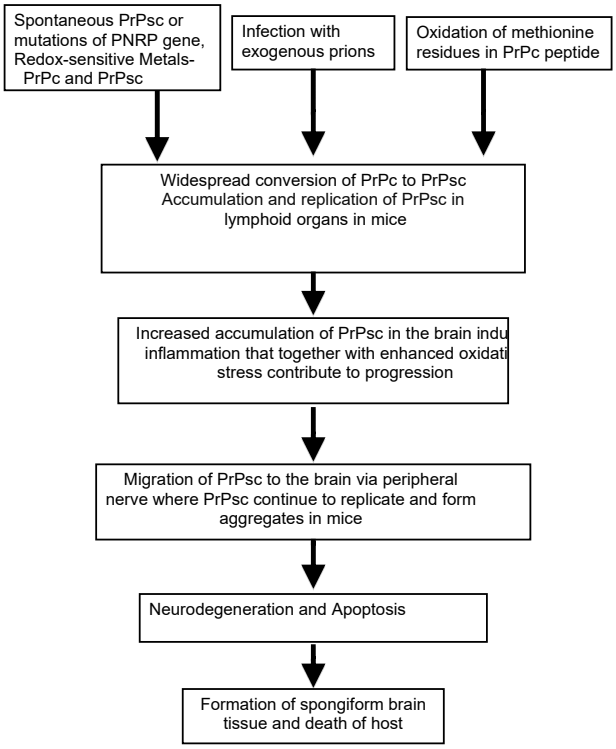

Fig. (1). Onset and progression of prion neurotoxicity.

of oxidative damage in regions of the brain infected with the PrPsc [6]. In patients with CJD, the levels of lipid peroxidation are increased in the Cerebral Spinal Fluid (CSF) and plasma, while the levels of polyunsaturated fatty acids are decreased in the plasma. In addition, the levels of ascorbate are reduced in both plasma and CSF, and alpha-tocopherol levels decreased in the CSF. Serum levels of the antioxidant uric acid are also decreased in sCJD [52]. Thus, increased oxidative stress may play an important role in the pathogenesis of CJD [53].

10.1. Oxidation of Methionine Residues in PrPc

Oxidation of methionine residues in PrPc may be responsible for conformational change from the α-helical form of PrPc to beta sheets of PrPsc and this may be a factor in regulating the onset and progression of familial CJD [5]. Oxidation of methionine 213 (Met213) and Met 205/206 converted PrPc to a PrPsc [5]. Oxidation of PrPc destabilizes the α–helical core of PrPc (Met205, Met212, Val209, Val160 and Tyr156), and this can facilitate the conversion of PrPc to PrPsc [54]. Loss of antioxidant defense systems may then contribute to the development and progression of prion disease [55]. In Fatal Familial Insomnia (FFI), associated with mutation in the D178N/129M gene, methionine oxidation also converts PrPc to a PrPsc [56]. Oxidation of methionine residues (Met 206 and Met 213) in Helix-3 appears to be an early biochemical defect that allows the conversion of PrPc to protease resistant PrPsc in familial CJD [57, 58]. Mutations in the G114v and A116V genes lie in the hydrophobic domain of PrPc. Cells expressing these mutations when exposed to PrPsc result in formation of relatively a protease digestible PrPsc structure that is still highly neurotoxic. PrPsc can be infective even when not protease-resistant [59]. Thus increased oxidative stress may be one of the early events in conversion of PrPc to PrPsc. Additional studies are needed to confirm a causal role for increased oxidative stress in the initiation and progression of prion disease.

11. PrPsc-INDUCED INFLAMMATION IN PRION DISEASE

Increased accumulation of PrPsc causes inflammatory events leading to dysfunctional neurons and eventually neuronal death [7]. In the PrPsc infected brain, activated microglia accumulate in the vicinity of abnormal prion aggregates. They release inflammatory cytokines such as IL-1β that are likely to play an important role in the pathogenesis of prion disease [8]. The role of microglia in inducing inflammatory events within the brain in prion disease has been recently reviewed [60]. The distribution of PrPsc in the neurons, astroglia, and microglia in the brain is related to the type of the originating PrPsc strain. Strain 22L of PrPsc primarily accumulates in the astroglia, whereas strain ME7 is mainly localized in the neurons and neuropil [61]. In the preceding report, expression of all 90 genes that regulate neuroinflammation were found to be upregulated in all three strains of PrPsc tested. This correlated with the activation of both astroglia and microglia that occurs in the early phase of the disease prior to the development of vascular pathology or clinical symptoms. Aggregated PrPsc induces inflammation and this is likely to contribute to spongiform degeneration of the brain [62]. Infection with PrPsc releases a pro-inflammatory cytokine IL-1β by activating the inflammasomes that by this means participate in the progression of the prion disease [63]. In CJD, such increased levels of IL-1β contribute to the death of neurons [64]. In human prion disease, inflammation-regulated expression of the micro RNA miR-146 was enhanced [65, 66]. In a puzzling contrast to the increased induction of inflammatory genes by PrPsc in the mouse brain, lymphoid tissues of sheep infected with PrPsc exhibit reduced expression of inflammatory genes [67]. Increased intracellular accumulation of Ca^{2+} is present in the brain of CJD, and activation of the calpain-cathepsin axis occurs at the pre-clinical stage of the disease [68]. It may then be that excessive levels of free ionic calcium might also have a role in the pathogenesis of this disease.

A synthetic peptide homologous to the region 106-126 of normal PrPc exhibits many features of PrPsc including the ability to produce apoptosis of neurons [69]. This peptide is toxic to the cells expressing PrPc, but not to the cells with the PrPc gene knocked out [70]. PrP 106-126 also enhances the expression of inducible Nitric Oxide (iNOS), and the levels of pro-inflammatory cytokines IL-1β and TNF-alpha, and activated NF-kB in mouse macrophages. Inhibition of NF-kB blocked these effects of PrP 106-126 on markers of inflammation [71].

In microglial culture, a PrPc fragment containing amino acids 90-231 also causes neuropathological changes similar to that produced by pathogenic prion protein PrPsc. This was preceded by microglial activation that led to release of prostaglandin E2 (PGE2) and Nitric Oxide (NO), in amounts toxic to neurons. When added to the mesencephalic neurons conditioned medium from PrP 90-231 treated microglia also induced degeneration. Celecoxib, an inhibitor of COX 2,

prevented PrP 90-231-induced activation of microglia, and release of PGE2 and NO. However, Ketoprofen (RS)-2-(3-benzoylphenyl)-propionic acid), a specific inhibitor of COX1 was ineffective [72]. These results suggest that PrPsc-induced inflammatory events make a significant contribution to the progression of prion disease in the brain.

The findings discussed above suggest that increased oxidative stress and chronic inflammation may be involved in the initiation and progression of prion disease.

12. MECHANISMS OF PRION NEUROTOXICITY

The mechanisms of prion neurotoxicity are highly complex. Studies on cell culture models showed that prion peptide PrP 106-126 induces neuropathology similar to that of PrPsc by multiple pathways. It activates microglia that release ROS and pro-inflammatory cytokines [73]. PrP 106-126 also increases Ca^{2+} uptake through voltage-sensitive Ca^{2+} channels, and this activates NMDA receptors leading to cell death [74]. Other mechanisms of toxicity were investigated by generating the β-sheet state of oligomeric PrPsc from recombinant full-length hamster, human, rabbit, and mutated rabbit PrPc, by shaking and sonication. These β-sheet oligomers are toxic to primary mouse cortical neurons independently of the presence of PrPc in the neurons. The mechanisms of toxicity produced by these beta-oligomers involve elevation of levels of pro-apoptotic proteins such as Bcl2, Bax, and caspase-3 [75]. A parallel mechanism has been suggested in the case of the beta-amyloid of Alzheimer's disease that has a beta-sheet configuration similar to that of PrPsc. This is suspected to cause neuronal death by the generation of pro-oxidant free radicals [76-78]. It is likely that aggregated misfolded PrPsc protein also induces neuronal death by this mechanism. This is further substantiated by reports, in which antioxidants prevent the progression of prion diseases in both cell culture and animals. This parallel between prion disease and Alzheimer's has been questioned because there may be an insufficient similarity between the β-sheet of Aβ peptides of AD and PrPsc [79].

PrPc acts as an antioxidant and loss of this activity in mutant PrPc may further increase the susceptibility of neurons to toxic insults [80]. The protective function of PrPc is substantiated by the finding that PrPc slowed neurodegeneration in transgenic mice expressing a pathogenic mutation of PrPc [81].

13. STUDIES WITH INDIVIDUAL ANTIOXIDANTS AND PHYTOCHEMICALS

Despite significant evidence for the role of increased oxidative stress and inflammation in the initiation and progression of prion disease, there are some reports, both *in vivo* and *ex vivo* on the potential utility of individual antioxidants and phytochemicals in delaying the onset and progression of the neurodegenerative changes.

Members of the peroxiredoxin class of enzymes have antioxidant properties and Peroxiredoxin 6 (Prdx6) protects human neuroblastoma cells (SK-N-SH) against oxidative stress caused by H_2O_2, hydroperoxides, or peroxynitrite [12]. In mice infected with prion disease, the overexpression of the Prdx6 gene protects against oxidative damage, reduces severity of behavioral deficits, and diminishes progression of neuropathology. Such overexpression increases the survival time in comparison to parallel infection of mice with knock-out of the Prdx6 gene [12].

Phytochemicals such as baicalein, the dried root of *Scutellaria baicalensis* (known as Huang-quin in traditional Chinese medicine) protect human neuroblastoma cells in culture against development of prion disease induced by the human PrPc Peptide106-126 (PrP-106-126). This peptide fragment induces neuropathological changes similar to those produced by PrPsc. The protective effect of baicalein was attributed to inhibition of ROS and restoration of mitochondrial functions [13].

Treatment with melatonin prevented PrP 106-126-induced damage to human neuroblastoma cells (SH-SY5Y). Melatonin activates beta-catenin and this may account for some of its antioxidant activity. An inhibitor of beta-catenin blocked the protective effect of melatonin [82].

Resveratrol has both antioxidant and anti-inflammatory activity. Treatment of neuronal cells with resveratrol attenuates PrP 106-126 induced cell death by activating autophagy that prevents mitochondrial dysfunction by inhibiting translocation of pro-apoptotic protein Bax to the mitochondria and cytochrome C release [83]. Resveratrol treatment also prevents PrP106-126-induced neuronal death by activating SIRT1 [84].

Rutin (quercetin-3-O-rutinoside) is a bioflavonoid known to possess antioxidant and anti-inflammatory activity. Treatment of dopaminergic neurons with rutin prevents PrP106-126-induced neuronal death by increasing the production of neurotropic factors and inhibiting activation of apoptotic pathways [85].

An extract of pomegranate seed oil in nanodroplet form that exhibits antioxidant activity delayed the manifestation of prion disease when administered to an asymptomatic genetic mouse model of prion disease [14]. This oil is rich in anthocyanins, phytosterols and ω-5 fatty acids but the contribution of each individual constituent was not evaluated.

Treatment of neuronal PC12 cells in culture with toxic PrP 106-126 peptide decreases intracellular levels of glutathione, superoxide dismutase activity, depolarizes the mitochondrial membrane, and increases the activity of caspase-3. These effects are all reduced in the presence of a synthetic antioxidant edaravone, 5-methyl-2-phenyl-4*H*-pyrazol-3-one [86].

Epigallocatechin Gallate (EGCG) and gallocatechin gallate are the primary polyphenols in green tea and are antioxidants. In scrapie-infected cells, treatment with EGCG prevents proliferation of abnormal prion configurations [87]. EGCG thus appears to have the potential to block the progression of prion disease.

Treatment of a mouse model of prion disease with a potent Mn-SOD/catalase mimetic, EUK-189, a salen-manganese complex, improves survival time and this is correlated with reduced oxidative and nitrosylative events and lessened spongiform changes [15].

Administration of Dimethylsulfoxide (DMSO) a solvent that exhibits antioxidant activity, to scrapie-infected ham-

sters significantly prolongs the period of disease latency, and delays the accumulation of PrPsc-induced aggregates in the brain [88].

These examples provide indirect support for the role of increased oxidative stress and inflammation in the development and progression of neurodegeneration in prion disease. Hence, reducing these cellular defects may reduce the rate of neurodegeneration caused by these diseases. This is substantiated by the protective effects of various antioxidants against the neurotoxicity caused by PrPsc or PrPc toxic peptide fragments. No studies have been performed in human prion diseases. However, in other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, the use of single antioxidants has produced inconsistent results varying from no effects to minimal transient benefits in clinical outcomes, although animal and cell culture studies produce consistent benefits. We propose that it is essential to simultaneously reduce oxidative stress and inflammation in order to maximize the benefit of remediation. This is best achieved by synchronized enhancement of the levels of antioxidant enzymes together with administration of anti-inflammatory and antioxidant compounds [89, 90].

14. POTENTIAL EXPLANATIONS FOR INCONSISTENT RESULTS RESULTING FROM TREATMENT OF NEURODEGENERATIVE DISEASES WITH SINGLE ANTIOXIDANTS

The reasons for individual antioxidants and phytochemicals failing to produce consistent results in human neurodegenerative diseases are not known; however, some potential causes are listed here: (a) antioxidants show differential subcellular distribution and different mechanisms of action; therefore, a single antioxidant cannot protect all parts of the cell; (b) a single antioxidant in a high internal oxidative environment in high-risk patients is oxidized and can then itself act as a pro-oxidant rather than as an antioxidant; (c) the protects against oxidative damage by elevating antioxidant enzymes and dietary and endogenous antioxidants; therefore, they all must be elevated, (d) antioxidants neutralize free radicals by donating electrons to those molecules with unpaired electron, whereas antioxidant enzymes destroy free radicals by catalysis, converting them to harmless molecules such as water and oxygen. Therefore, both of these agents may need to be enhanced to achieve substantial protection against oxidative damage; (e) the affinity of different antioxidants for free radicals differs, depending upon their solubility; (f) both the aqueous and lipid compartments of the cell need to be protected together. Water-soluble antioxidants such as vitamin C and glutathione protect molecules in the aqueous environment of the cells, whereas lipid-soluble antioxidants such as vitamin A and vitamin E protect molecules in the lipid compartment; (g) vitamin E is more effective in quenching free radicals in a reduced oxygenated cellular environment, whereas vitamin C and alpha-tocopherol are more effective in a higher oxygenated environment of the cells [91]; (h) vitamin C is important for recycling the oxidized form of alpha-tocopherol to the antioxidant form [92]; (i) Various antioxidants produce protective proteins by altering the expression of a distinctive suite of different microRNAs; [93]. For example, some antioxidants can activate Nrf2 by upregulating miR-200a that inhibits its target protein Keap1, whereas others activate Nrf2 by downregulating miR-21 that binds with 3'-UTR Nrf2 mRNA [94].

Due to these considerations, the use of a single antioxidant cannot be expected to produce optimal protection against all the oxidative and inflammatory processes, which contribute to the progression of prion disease. It is therefore proposed that the best means of restoring the most favorable intracellular state, involves using a range of dietary and endogenous antioxidants additions, in concert. While oral supplementation can increase the levels of antioxidants within the cell, elevation of the levels of antioxidant enzymes requires activation of specific transcription factors, especially Nrf2. Understanding the regulation of Nrf2 activation is thus significant in amplifying the therapeutic concepts discussed above.

15. ACTIVATION OF Nrf2

15.1. Nrf2

The nuclear transcriptional factor, Nrf2 (nuclear factor-erythroid-2- related factor 2) belongs to the Cap ´N´Collar (CNC) family that contains a conserved basic leucine zipper (bZIP) transcriptional factor [95]. Under physiological conditions, Nrf2 is associated with Kelch-like ECH associated protein 1 (Keap1), which acts as an inhibitor of Nrf2 [96]. Keap1 protein serves as an adaptor to link Nrf2 to the ubiquitin ligase CuI-Rbx1 complex for degradation by proteasomes and maintains the steady levels of Nrf2 in the cytoplasm. Nrf2-keap1 complex is primarily located in the cytoplasm; Keap1 acts as a sensor for ROS/electrophilic stress.

15.2. Activation of Nrf2 During Acute Oxidative Stress

During acute oxidative stress, ROS activate Nrf2 which then dissociates itself from Keap1- CuI-Rbx1 complex and translocates in the nucleus where it heterodimerizes with a small Maf protein, binds with ARE leading to increased expression of target genes coding for several cytoprotective enzymes including antioxidant enzymes [97-99].

15.3. Failure to Activate Nrf2 During Chronic Oxidative Stress

During extended chronic oxidative stress, Nrf2 becomes resistant to ROS [100-102], suggesting that activation of Nrf2 by a ROS-independent mechanism exists. This is evidenced by the fact that increased oxidative stress occurs despite the presence of Nrf2 in prion disease. The question arises as to how to activate ROS-resistant Nrf2 in prion disease.

15.4. Antioxidants and Phytochemicals Activate ROS-Resistant Nrf2

Some examples are vitamin E and genistein [8], alpha-lipoic acid [103], curcumin [104], resveratrol [105, 106], omega-3-fatty acids, [107, 108], glutathione [109], NAC [110], and coenzyme Q10 [111]. Several plant-derived phytochemicals, such as epigallocatechin-3-gallate, carestol, kahweol, cinnamonyl-based compounds, zerumbone, lycopene and carnosol [95, 112, 113], genistein [8], allicin, a major organosulfur compound found in garlic [71], sulforaphane, a organosulfur compound, found in cruciferous vegetables [114], and kavalactones (methysticin, kavain and yangonin) [115].

15.5. Binding of Nrf2 with the Antioxidant Response Elements (ARE) in the Nucleus

An activation of Nrf2 alone is not sufficient to increase the levels of antioxidant enzymes. AREs are gene promoters that mediate the transcriptional induction of a battery of genes which comprise much of the chemoprotective response system. Nrf2 binds to and activates this promoter and thus increases the expression of target genes coding for a suite of antioxidant enzymes. The binding ability of Nrf2 to ARE was impaired in aged rats and this defect was restored by supplementation with alpha-lipoic acid [103]. The issue as to whether the binding ability of Nrf2 with ARE is impaired in prion disease is of interest but remains unknown.

16. REDUCTION OF CHRONIC INFLAMMATION

Activation of Nrf2 also suppresses chronic inflammation [116, 117]. Many antioxidant compounds also reduce inflammation [118-123] suggesting that these two adverse events are closely linked.

17. PROPOSED MIXTURE OF MICRONUTRIENTS IN THE MANAGEMENT OF PRION DISEASE

Because each antioxidant exhibits differing sub-cellular distribution, various mechanisms of action, preferential affinity for diverse types of free radicals a mixture of micronutrients containing vitamin A, mixed carotenoids, vitamin C, alpha-tocopheryl acetate, a-tocopheryl succinate, vitamin D3, alpha-lipoic acid, n-acetyl cysteine, coenzyme Q10, L-carnitine, omega-3-fatty acids, curcumin, resveratrol, all B-vitamins, selenomethionine, and zinc is proposed. This mixture would increase the levels of antioxidant enzymes by activating the Nrf2 pathway and enhancing the levels of dietary and endogenous antioxidant compounds, which could lead to simultaneously reduction in oxidative stress and chronic inflammation in prion disease. Many of these agents also activate an Nrf2 pathway that does not respond directly to oxidative stress. These allows them to act as an antioxidant by several distinct mechanisms.

There are no effective strategies for delaying the progression of prion diseases. Although the use of single antioxidants has protected against neutoxicity of aggregated misfolded prion proteins in cell culture and animal models, a single agent cannot elevate antioxidant status in all cell compartments and induce antioxidant enzymes all at the same time. The suggested micronutrient mixture may reduce the rate of progression prion disease in individuals who have been infected with PrPsc but have not developed the symptoms of the disease. This mixture of micronutrients, in combination with standard care, may also be useful in decreasing the rate of progression of the disease. Pre-clinical and clinical studies are needed to substantiate this potential role of such a mixture of micronutrients in reducing the rate of progression of prion disease.

CONCLUSION

Prion diseases are a group of transmissible incurable progressive fatal neurodegenerative diseases. They are caused by an infective aggregated misfolded isoform of the cellular protein (PrPsc.) that induces Transmissible Spongiform Encephalopathy (TSE). Studies suggest that increased oxidative stress is one of the factors that initiate conversion of PrPc to PrPsc. Redox-labile metals Cu and Fe bound with PrPsc may promote neurodegeneration by increased Fenton cycling leading to excessive production of free radicals. From the studies in mice, it appears that some phagocytes may help disseminate PrPsc of exogenous origin by facilitating their entry into lymphoid tissues where they propagate. In contrast, other types of phagocyte may remove infective peptides by phagocytosis. Since administration of individual single antioxidant produces inconsistent results varying from no effects to transient beneficial effects on clinical outcomes in other neurodegenerative diseases, a mixture of micronutrients that can simultaneously reduce oxidative stress and inflammation in prion disease is proposed. This mixture of micronutrients alone or in combination with standard care may reduce the progression of prion disease.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Gajdusek DC, Zigas V. Degenerative disease of the central nervous system in New Guinea; the endemic occurrence of kuru in the native population. N Engl J Med 1957; 257(20): 974-8.

[2] Gibbs CJ, Gajdusek DC, Asher DM, *et al*. Creutzfeldt-Jakob disease (spongiform encephalopathy): Transmission to the chimpanzee. Science 1968; 161(3839): 388-9.

[3] Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216(4542): 136-44.

[4] Prusiner SB. Cell biology. A unifying role for prions in neurodegenerative diseases. Science 2012; 336(6088): 1511-3.

[5] Chen YR, Yi FF, Li XY, *et al*. Resveratrol attenuates ventricular arrhythmias and improves the long-term survival in rats with myocardial infarction. Cardiovasc Drugs Ther 2008; 22(6): 479-85.

[6] Brazier MW, Lewis V, Ciccotosto GD, *et al*. Correlative studies support lipid peroxidation is linked to PrP(res) propagation as an early primary pathogenic event in prion disease. Brain Res Bull 2006; 68(5): 346-54.

[7] Crespo I, Roomp K, Jurkowski W, *et al*. Gene regulatory network analysis supports inflammation as a key neurodegeneration process in prion disease. BMC Syst Biol 2012; 6: 132.

[8] Algarzae N, Hebron M, Miessau M, *et al*. Parkin prevents cortical atrophy and Abeta-induced alterations of brain metabolism: (1)(3)C NMR and magnetic resonance imaging studies in AD models. Neuroscience 2012; 225: 22-34.

[9] Carroll JA, Striebel JF, Race B, *et al*. Prion infection of mouse brain reveals multiple new upregulated genes involved in neuroinflammation or signal transduction. J Virol 2015; 89(4): 2388-404.

[10] Mustafa AG, Singh IN, Wang J, *et al*. Mitochondrial protection after traumatic brain injury by scavenging lipid peroxyl radicals. J Neurochem 2010; 114(1): 271-80.

[11] Yen CF, Harischandra DS, Kanthasamy A, *et al*. Copper-induced structural conversion templates prion protein oligomerization and neurotoxicity. Sci Adv 2016; 2(7): e1600014.

[12] Asuni AA, Guridi M, Sanchez S, *et al*. Antioxidant peroxiredoxin 6 protein rescues toxicity due to oxidative stress and cellular hypoxia *in vitro*, and attenuates prion-related pathology *in vivo*. Neurochem Int 2015; 90: 152-65.

[13] Lee ST, Chu K, Jung KH, *et al*. Altered expression of miR-202 in cerebellum of multiple-system atrophy. Mol Neurobiol 2015; 51(1): 180-6.

[14] Mizrahi M, Friedman-Levi Y, Larush L, *et al*. Pomegranate seed oil nanoemulsions for the prevention and treatment of neurodegenerative diseases: The case of genetic CJD. Nanomedicine 2014; 10(6): 1353-63.

[15] Brazier MW, Doctrow SR, Masters CL, *et al*. A manganese-superoxide dismutase/catalase mimetic extends survival in a mouse model of human prion disease. Free Radic Biol Med 2008; 45(2): 184-92.

[16] Holman RC, Belay ED, Christensen KY, *et al*. Human prion diseases in the United States. PLoS One 2010; 5(1): e8521.

[17] Parchi P, Giese A, Capellari S, *et al*. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46(2): 224-33.

[18] Hsiao K, Meiner Z, Kahana E, *et al*. Mutation of the prion protein in Libyan Jews with Creutzfeldt-Jakob disease. N Engl J Med 1991; 324(16): 1091-7.

[19] Meiner Z, Gabizon R, Prusiner SB. Familial Creutzfeldt-Jakob disease. Codon 200 prion disease in Libyan Jews. Medicine (Baltimore) 1997; 76(4): 227-37.

[20] Belay ED, Sejvar JJ, Shieh WJ, *et al*. Variant Creutzfeldt-Jakob disease death, United States. Emerg Infect Dis 2005; 11(9): 1351-4.

[21] Ghosh S. Mechanism of intestinal entry of infectious prion protein in the pathogenesis of variant Creutzfeldt-Jakob disease. Adv Drug Deliv Rev 2004; 56(6): 915-20.

[22] Belay ED. Transmissible spongiform encephalopathies in humans. Annu Rev Microbiol 1999; 53: 283-314.

[23] Valleron AJ, Boelle PY, Will R, *et al*. Estimation of epidemic size and incubation time based on age characteristics of vCJD in the United Kingdom. Science 2001; 294(5547): 1726-8.

[24] Armstrong RA, Cairrns NJ, Lantos PL. Quantification of the vacuolation (spongiform change) and prion protein deposition in 11 patients with s[oradic Creutzfeldt-Jakob disease. Acta Neuropathol 2001; 102: 591-6.

[25] Iwasaki Y. Creutzfeldt-Jakob disease. Neuropathology 2017; 37(2): 174-88.

[26] Bradford BM, Crocker PR, Mabbott NA. Peripheral prion disease pathogenesis is unaltered in the absence of sialoadhesin (Siglec-1/CD169). Immunology 2014; 143(1): 120-9.

[27] Prinz M, Heikenwalder M, Junt T, *et al*. Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion. Nature 2003; 425(6961): 957-62.

[28] Prinz M, Montrasio F, Klein MA, *et al*. Lymph nodal prion replication and neuroinvasion in mice devoid of follicular dendritic cells. Proc Natl Acad Sci U S A 2002; 99(2): 919-24.

[29] Grassmann A, Wolf H, Hofmann J, *et al*. Cellular aspects of prion replication *in vitro*. Viruses 2013; 5(1): 374-405.

[30] Basler K, Oesch B, Scott M, *et al*. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell 1986; 46(3): 417-28.

[31] Chesebro B, Race R, Wehrly K, *et al*. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. Nature 1985; 315(6017): 331-3.

[32] Rigter A, Priem J, Timmers-Parohi D, *et al*. Prion protein self-peptides modulate prion interactions and conversion. BMC Biochem 2009; 10: 29.

[33] Ashok A, Hegde RS. Selective processing and metabolism of disease-causing mutant prion proteins. PLoS Pathog 2009; 5(6): e1000479.

[34] Bossers A, Belt P, Raymond GJ, *et al*. Scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of sheep prion protein to protease-resistant forms. Proc Natl Acad Sci U S A 1997; 94(10): 4931-6.

[35] Sabuncu E, Petit S, Le Dur A, *et al*. PrP polymorphisms tightly control sheep prion replication in cultured cells. J Virol 2003; 77(4): 2696-700.

[36] Kobayashi A, Teruya K, Matsuura Y, *et al*. The influence of PRNP polymorphisms on human prion disease susceptibility: An update. Acta Neuropathol 2015; 130(2): 159-70.

[37] Apetri AC, Vanik DL, Surewicz WK. Polymorphism at residue 129 modulates the conformational conversion of the D178N variant of human prion protein 90-231. Biochemistry 2005; 44(48): 15880-8.

[38] Nadal RC, Abdelraheim SR, Brazier MW, *et al*. Prion protein does not redox-silence Cu^{2+}, but is a sacrificial quencher of hydroxyl radicals. Free Radic Biol Med 2007; 42(1): 79-89.

[39] Basu S, Mohan ML, Luo X, *et al*. Modulation of proteinase K-resistant prion protein in cells and infectious brain homogenate by redox iron: Implications for prion replication and disease pathogenesis. Mol Biol Cell 2007; 18(9): 3302-12.

[40] Kretzschmar HA, Tings T, Madlung A, *et al*. Function of PrP(C) as a copper-binding protein at the synapse. Arch Virol Suppl 2000; 16: 239-49.

[41] Millhauser GL. Copper and the prion protein: Methods, structures, function, and disease. Annu Rev Phys Chem 2007; 58: 299-320.

[42] Kamboj SS, Chopra K, Sandhir R. Hyperglycemia-induced alterations in synaptosomal membrane fluidity and activity of membrane bound enzymes: Beneficial effect of N-acetylcysteine supplementation. Neuroscience 2009; 162(2): 349-58.

[43] Kawano T. Prion-derived copper-binding peptide fragments catalyze the generation of superoxide anion in the presence of aromatic monoamines. Int J Biol Sci 2007; 3: 57-63.

[44] Aarsland D, Rongve A, Nore SP, *et al*. Frequency and case identification of dementia with Lewy bodies using the revised consensus criteria. Dement Geriatr Cogn Disord 2008; 26(5): 445-52.

[45] Vilette D, Laulagnier K, Huor A, *et al*. Efficient inhibition of infectious prions multiplication and release by targeting the exosomal pathway. Cell Mol Life Sci 2015; 72(22): 4409-27.

[46] Guo BB, Bellingham SA, Hill AF. Stimulating the release of exosomes increases the intercellular transfer of prions. J Biol Chem 2016; 291(10): 5128-37.

[47] Lastres-Becker I. Role of the transcription factor Nrf2 in Parkinson's disease: New Insights. J Alzheimers Dis Parkinsonism 2017; 7(4): 1-9.

[48] Vella LJ, Sharples RA, Lawson VA, *et al*. Packaging of prions into exosomes is associated with a novel pathway of PrP processing. J Pathol 2007; 211(5): 582-90.

[49] Fevrier B, Vilette D, Archer F, *et al*. Cells release prions in association with exosomes. Proc Natl Acad Sci U S A 2004; 101(26): 9683-8.

[50] Mays CE, Ryou C. Plasminogen: A cellular protein cofactor for PrPSc propagation. Prion 2011; 5(1): 22-7.

[51] Kovacs GG, Seguin J, Quadrio I, *et al*. Genetic Creutzfeldt-Jakob disease associated with the E200K mutation: Characterization of a complex proteinopathy. Acta Neuropathol 2011; 121(1): 39-57.

[52] Chen PY, Hou CW, Shibu MA, *et al*. Protective effect of Co-enzyme Q10 On doxorubicin-induced cardiomyopathy of rat hearts. Environ Toxicol 2017; 32(2): 679-89.

[53] Arlt S, Kontush A, Zerr I, *et al*. Increased lipid peroxidation in cerebrospinal fluid and plasma from patients with Creutzfeldt-Jakob disease. Neurobiol Dis 2002; 10(2): 150-6.

[54] Younan ND, Nadal RC, Davies P, *et al*. Methionine oxidation perturbs the structural core of the prion protein and suggests a generic misfolding pathway. J Biol Chem 2012; 287(34): 28263-75.

[55] Brown DR. Neurodegeneration and oxidative stress: Prion disease results from loss of antioxidant defence. Folia Neuropathol 2005; 43(4): 229-43.

[56] Che H, Sun LH, Guo F, *et al*. Expression of amyloid-associated miRNAs in both the forebrain cortex and hippocampus of middle-aged rat. Cell Physiol Biochem 2014; 33(1): 11-22.

[57] Canello T, Frid K, Gabizon R, *et al*. Oxidation of Helix-3 methionines precedes the formation of PK resistant PrP. PLoS Pathog 2010; 6(7): e1000977.

[58] Colombo G, Meli M, Morra G, *et al*. Methionine sulfoxides on prion protein Helix-3 switch on the alpha-fold destabilization required for conversion. PLoS One 2009; 4(1): e4296.

[59] Coleman BM, Harrison CF, Guo B, *et al*. Pathogenic mutations within the hydrophobic domain of the prion protein lead to the formation of protease-sensitive prion species with increased lethality. J Virol 2014; 88(5): 2690-703.

[60] Obst J, Simon E, Mancuso R, *et al*. The role of microglia in prion diseases: A paradigm of functional diversity. Front Aging Neurosci 2017; 9: 207.

[61] Carroll JA, Striebel JF, Rangel A, *et al*. Prion strain differences in accumulation of PrPSc on neurons and glia are associated with similar expression profiles of neuroinflammatory genes: comparison of three prion strains. PLoS Pathog 2016; 12(4): e1005551.

[62] Absalon S, Kochanek DM, Raghavan V, *et al*. MiR-26b, upregulated in Alzheimer's disease, activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons. J Neurosci 2013; 33(37): 14645-59.

[63] Alam MM, Iqbal S, Naseem I. Ameliorative effect of riboflavin on hyperglycemia, oxidative stress and DNA damage in type-2 diabetic mice: Mechanistic and therapeutic strategies. Arch Biochem Biophys 2015; 584: 10-9.

[64] Van Everbroeck B, Dewulf E, Pals P, *et al*. The role of cytokines, astrocytes, microglia and apoptosis in Creutzfeldt-Jakob disease. Neurobiol Aging 2002; 23(1): 59-64.

[65] Choi SH, Aid S, Caracciolo L, *et al*. Cyclooxygenase-1 inhibition reduces amyloid pathology and improves memory deficits in a mouse model of Alzheimer's disease. J Neurochem 2013; 124(1): 59-68.

[66] Li YY, Cui JG, Hill JM, *et al*. Increased expression of miRNA-146a in Alzheimer's disease transgenic mouse models. Neuroscience letters 2011; 487(1): 94-8.

[67] Gossner AG, Hopkins J. The effect of PrP(Sc) accumulation on inflammatory gene expression within sheep peripheral lymphoid tissue. Vet Microbiol 2015; 181(3-4): 204-11.

[68] Llorens F, Thune K, Sikorska B, *et al*. Altered Ca(2+) homeostasis induces Calpain-Cathepsin axis activation in sporadic Creutzfeldt-Jakob disease. Acta Neuropathol Commun 2017; 5(1): 35.

[69] Forloni G, Angeretti N, Chiesa R, *et al*. Neurotoxicity of a prion protein fragment. Nature 1993; 362(6420): 543-6.

[70] Fioriti L, Quaglio E, Massignan T, *et al*. The neurotoxicity of prion protein (PrP) peptide 106-126 is independent of the expression level of PrP and is not mediated by abnormal PrP species. Mol Cell Neurosci 2005; 28(1): 165-76.

[71] Ahmad A, Crupi R, Impellizzeri D, *et al*. Administration of palmitoylethanolamide (PEA) protects the neurovascular unit and reduces secondary injury after traumatic brain injury in mice. Brain Behav Immun 2012; 26(8): 1310-21.

[72] Sanchez-Rodriguez C, Martin-Sanz E, Cuadrado E, *et al*. Protective effect of polyphenols on presbycusis *via* oxidative/nitrosative stress suppression in rats. Exp Gerontol 2016; 83: 31-6.

[73] Beck AT, Steer RA, Ball R, *et al*. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. J Pers Assess 1996; 67(3): 588-97.

[74] Brown DR, Herms JW, Schmidt B, *et al*. PrP and beta-amyloid fragments activate different neurotoxic mechanisms in cultured mouse cells. Eur J Neurosci 1997; 9(6): 1162-9.

[75] Christensen KE, Mikael LG, Leung KY, *et al*. High folic acid consumption leads to pseudo-MTHFR deficiency, altered lipid metabolism, and liver injury in mice. Am J Clin Nutr 2015; 101(3): 646-58.

[76] Butterfield DA, Hensley K, Harris M, *et al*. Beta-Amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: Implications to Alzheimer's disease. Biochem Biophys Res Commun 1994; 200(2): 710-5.

[77] Butterfield DA, Bush AI. Alzheimer's amyloid beta-peptide (1-42): Involvement of methionine residue 35 in the oxidative stress and neurotoxicity properties of this peptide. Neurobiol Aging 2004; 25(5): 563-8.

[78] Varadarajan S, Yatin S, Kanski J, *et al*. Methionine residue 35 is important in amyloid beta-peptide-associated free radical oxidative stress. Brain Res Bull 1999; 50(2): 133-41.

[79] Lahiri DK. Prions: A piece of the puzzle? Science 2012; 337(6099): 1172.

[80] Wong BS, Pan T, Liu T, *et al*. Prion disease: A loss of antioxidant function? Biochem Biophys Res Commun 2000; 275(2): 249-52.

[81] Telling GC, Haga T, Torchia M, *et al*. Interactions between wild-type and mutant prion proteins modulate neurodegeneration in transgenic mice. Genes Dev 1996; 10(14): 1736-50.

[82] Im N, Zhou W, Na M, *et al*. Pierisformoside B exhibits neuroprotective and anti-inflammatory effects in murine hippocampal and microglial cells *via* the HO-1/Nrf2-mediated pathway. Int Immunopharmacol 2014; 24(2): 353-60.

[83] Jeong JK, Moon MH, Bae BC, *et al*. Autophagy induced by resveratrol prevents human prion protein-mediated neurotoxicity. Neurosci Res 2012; 73(2): 99-105.

[84] Cho HJ, Liu G, Jin SM, *et al*. MicroRNA-205 regulates the expression of Parkinson's disease-related leucine-rich repeat kinase 2 protein. Hum Mol Genet 2013; 22(3): 608-20.

[85] Albanes D, Till C, Klein EA, *et al*. Plasma tocopherols and risk of prostate cancer in the Selenium and Vitamin E Cancer Prevention Trial (SELECT). Cancer Prev Res (Phila) 2014; 7(9): 886-95.

[86] Ahn JH, Yoo MH, Lee HJ, *et al*. Coenzyme Q10 in combination with steroid therapy for treatment of sudden sensorineural hearing loss: A controlled prospective study. Clin Otolaryngol 2010; 35(6): 486-9.

[87] Rambold AS, Miesbauer M, Olschewski D, *et al*. Green tea extracts interfere with the stress-protective activity of PrP and the formation of PrP. J Neurochem 2008; 107(1): 218-29.

[88] Shaked GM, Engelstein R, Avraham I, *et al*. Dimethyl sulfoxide delays PrP sc accumulation and disease symptoms in prion-infected hamsters. Brain Res 2003; 983(1-2): 137-43.

[89] Prasad KN, Bondy SC. Inhibition of early upstream events in prodromal Alzheimer's disease by use of targeted antioxidants. Curr Aging Sci 2014; 7(2): 77-90.

[90] Prasad KN. Simultaneous activation of Nrf2 and elevation of antioxidant compounds for reducing oxidative stress and chronic inflammation in human Alzheimer's disease. Mech Ageing Dev 2016; 153: 41-7.

[91] Vile GF, Winterbourn CC. Inhibition of adriamycin-promoted microsomal lipid peroxidation by beta-carotene, alpha-tocopherol and retinol at high and low oxygen partial pressures. FEBS Lett 1988; 238(2): 353-6.

[92] Niki E. Interaction of ascorbate and alpha-tocopherol. Ann N Y Acad Sci 1987; 498: 186-99.

[93] Prasad KN. Oxidative stress and pro-inflammatory cytokines may act as one of the signals for regulating microRNAs expression in Alzheimer's disease. Mech Ageing Dev 2017; 162: 63-71.

[94] Wu H, Kong L, Tan Y, *et al*. C66 ameliorates diabetic nephropathy in mice by both upregulating NRF2 function via increase in miR-200a and inhibiting miR-21. Diabetologia 2016; 59(7): 1558-68.

[95] Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev 2013; 27(20): 2179-91.

[96] Williamson TP, Johnson DA, Johnson JA. Activation of the Nrf2-ARE pathway by siRNA knockdown of Keap1 reduces oxidative stress and provides partial protection from MPTP-mediated neurotoxicity. Neurotoxicology 2012; 33(3): 272-9.

[97] Hashimoto M, Yoshimoto M, Sisk A, *et al*. NACP, a synaptic protein involved in Alzheimer's disease, is differentially regulated during megakaryocyte differentiation. Biochem Biophys Res Commun 1997; 237(3): 611-6.

[98] Kirsh VA, Hayes RB, Mayne ST, *et al*. Supplemental and dietary vitamin E, beta-carotene, and vitamin C intakes and prostate cancer risk. J Natl Cancer Inst 2006; 98(4): 245-54.

[99] Albini A, Morini M, D'Agostini F, *et al*. Inhibition of angiogenesis-driven Kaposi's sarcoma tumor growth in nude mice by oral N-acetylcysteine. Cancer Res 2001; 61(22): 8171-8.

[100] Ramsey CP, Glass CA, Montgomery MB, *et al*. Expression of Nrf2 in neurodegenerative diseases. J Neuropathol Exp Neurol 2007; 66(1): 75-85.

[101] Chen PC, Vargas MR, Pani AK, *et al*. Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. Proc Natl Acad Sci U S A 2009; 106(8): 2933-8.

[102] Lastres-Becker I, Ulusoy A, Innamorato NG, *et al*. alpha-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson's disease. Hum Mol Genet 2012; 21(14): 3173-92.

[103] Iwanaga M, Hsu WL, Soda M, *et al*. Risk of myelodysplastic syndromes in people exposed to ionizing radiation: a retrospective cohort study of Nagasaki atomic bomb survivors. J Clin Oncol 2011; 29(4): 428-34.

[104] Trujillo J, Chirino YI, Molina-Jijon E, *et al*. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol 2013; 1: 448-56.

[105] Steele ML, Fuller S, Patel M, *et al*. Effect of Nrf2 activators on release of glutathione, cysteinylglycine and homocysteine by human U373 astroglial cells. Redox Biol 2013; 1: 441- 5.

[106] Kode A, Rajendrasozhan S, Caito S, *et al*. Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. Am J Physiol Lung Cell Mol Physiol 2008; 294(3): L478-88.

[107] Gao L, Wang J, Sekhar KR, *et al*. Novel n-3 fatty acid oxidation products activate Nrf2 by destabilizing the association between Keap1 and Cullin3. J Biol Chem 2007; 282(4): 2529-37.

[108] Aoi J, Endo M, Kadomatsu T, *et al*. Angiopoietin-like protein 2 accelerates carcinogenesis by activating chronic inflammation and oxidative stress. Mol Cancer Res 2014; 12(2): 239-49.

[109] Song J, Kang SM, Lee WT, *et al*. Glutathione protects brain endothelial cells from hydrogen peroxide-induced oxidative stress by increasing nrf2 expression. Exp Neurobiol 2014; 23(1): 93-103.

[110] Atkinson J, Kapralov A, Huang Z, *et al*. Mitochondria-targeted ligands of hene-iron in cytochrome c as novel radioprotectors/radiomitigators. Radiat Res 2010, 56th Annual Meeting Radiation Research Society, Maui, Hawaii, Sept. 25-29 :58a.

[111] Beriat GK, Ezerarslan H, Akmansu SH, *et al*. Comparison of efficacy of different treatment methods in the treatment of idiopathic tinnitus. Kulak Burun Bogaz Ihtis Derg 2011; 21(3): 145-53.

[112] Bai H, Liu R, Chen HL, *et al*. Enhanced antioxidant effect of caffeic acid phenethyl ester and Trolox in combination against radiation induced-oxidative stress. Chem Biol Interact 2014; 207: 7-15.

[113] Cui L, Jeong H, Borovecki F, *et al*. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 2006; 127(1): 59-69.

[114] Bergstrom P, Andersson HC, Gao Y, *et al*. Repeated transient sulforaphane stimulation in astrocytes leads to prolonged Nrf2-mediated gene expression and protection from superoxide-induced damage. Neuropharmacology 2011; 60(2-3): 343-53.

[115] Wruck CJ, Gotz ME, Herdegen T, *et al*. Kavalactones protect neural cells against amyloid beta peptide-induced neurotoxicity via extracellular signal-regulated kinase 1/2-dependent nuclear factor erythroid 2-related factor 2 activation. Mol Pharmacol 2008; 73(6): 1785-95.

[116] Kim J, Cha YN, Surh YJ. A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. Mutat Res 2010; 690(1-2): 12-23.

[117] Li W, Khor TO, Xu C, *et al*. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. Biochem Pharmacol 2008; 76(11): 1485-9.

[118] Abate A, Yang G, Dennery PA, *et al*. Synergistic inhibition of cyclooxygenase-2 expression by vitamin E and aspirin. Free Radic Biol Med 2000; 29(11): 1135-42.

[119] Fu Y, Zheng S, Lin J, *et al*. Curcumin protects the rat liver from CCl4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Mol Pharmacol 2008; 73(2): 399-409.

[120] Lee HS, Jung KK, Cho JY, *et al*. Neuroprotective effect of curcumin is mainly mediated by blockade of microglial cell activation. Pharmazie 2007; 62(12): 937-42.

[121] Rahman S, Bhatia K, Khan AQ, *et al*. Topically applied vitamin E prevents massive cutaneous inflammatory and oxidative stress responses induced by double application of 12-O-tetradecanoylphorbol-13-acetate (TPA) in mice. Chem Biol Interact 2008; 172(3): 195-205.

[122] Suzuki YJ, Aggarwal BB, Packer L. Alpha-lipoic acid is a potent inhibitor of NF-kappa B activation in human T cells. Biochem Biophys Res Commun 1992; 189(3): 1709-15.

[123] Zhu J, Yong W, Wu X, *et al*. Anti-inflammatory effect of resveratrol on TNF-alpha-induced MCP-1 expression in adipocytes. Biochem Biophys Res Commun 2008; 369(2): 471-7.