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Forum Review

Alzheimer's Disease: A Lesson from Mitochondrial Dysfunction

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ABSTRACT

Extensive literature exists supporting a role for mitochondrial dysfunction and oxidative damage in the pathogenesis of Alzheimer's disease. Mitochondria are a major source of intracellular reactive oxygen species and are particularly vulnerable to oxidative stress. This review discusses evidence supporting the notion that mitochondrial dysfunction is intimately associated with Alzheimer's disease pathogenesis. Furthermore, the potential connection between mitochondrial dysfunction/oxidative stress and autophagy in Alzheimer's disease is also discussed. As a result of insufficient digestion of oxidatively damaged macromolecules and organelles by autophagy, neurons progressively accumulate lipofuscin (biological garbage) that could exacerbate neuronal dysfunction. The knowledge that mitochondrial dysfunction has a preponderant role in several pathological conditions instigated the development of mitochondrial antioxidant therapies. Mitochondria-targeted antioxidant treatments are briefly discussed in this review. *Antioxid. Redox Signal.* 9, 1621–1630.

INTRODUCTION

Alzheimer's disease (AD) is a progressive, degenerative brain disorder resulting in cognitive and behavioral decline and is the leading cause of dementia in the Western world. Two pathological hallmarks are observed in AD brains at autopsy: intracellular neurofibrillary tangles (NFT) and extracellular senile plaques (SP) in the neocortex, hippocampus, and other subcortical regions essential for cognitive function (58). NFT are formed from paired helical filaments composed of neurofilaments and hyperphosphorylated tau protein. In turn, plaque cores are formed mostly from deposition of amyloid β (Aβ) peptide that results from the cleavage of the amyloid β precursor protein (AβPP).

Oxidative stress is an important issue in understanding the pathogenesis of AD. Indeed, there is accumulating evidence suggesting that oxidative stress occurs prior to the onset of symptoms in AD and oxidative damage is found not only in the vulnerable regions of the brain affected in disease (44, 72), but also peripherally (34, 59, 78). Moreover, it has been shown that oxidative damage occurs before Aβ plaque formation (72).

The complex nature and genesis of oxidative damage in AD can now be partly answered by mitochondrial abnormalities that can initiate oxidative stress. Defective mitochondria are incapable of producing enough energy (ATP) and often generate increased amounts of reactive oxygen species (ROS), further enhancing oxidative stress. The increased levels of damaged cellular components are not completely turned over by macroautophagy (hereafter referred as autophagy) and other cellular repair systems, leading to a progressive accumulation of biological "garbage," such as defective mitochondria, cytoplasmic protein aggregates, and an intralysosomal undegradable material, lipofuscin. Along with oxidative damage and mitochondrial dysfunction, activation of autophagy has been reported to occur early in AD (71), implying that these early neuronal changes might be interrelated.

This review is mainly focused on the discussion of evidence supporting the involvement of mitochondria in AD pathogenesis. Then we will address the interplay between oxidative stress, mitochondrial abnormalities, and autophagy. Finally, we will briefly discuss antioxidant-based strategies aimed to improve and protect mitochondrial function.

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THE TWO FACES OF MITOCHONDRIA

Mitochondria are uniquely poised to play a pivotal role in neuronal cell survival or death after central nervous system (CNS) injury because they are regulators of both energy metabolism and apoptotic pathways (32, 33, 101). Maintaining mitochondrial homeostasis and bioenergetics in neurons is even more critical, due to their almost complete dependence on mitochondrial-derived ATP (15, 69, 93, 94). However, the production of energy is accompanied by the generation of ROS as by-products of the oxidative phosphorylation process. Inevitably, if the amount of ROS produced unbalances the few antioxidants, oxidative stress occurs, followed by neuronal damage. The CNS is particularly susceptible to ROS-induced damage (for review, see Ref. 40) because (a) it has a high consumption of oxygen; (b) it contains high levels of membrane polyunsaturated fatty acids susceptible to free radical attack; (c) it is relatively deficient in oxidative defences (poor catalase activity and moderate superoxide dismutase and glutathione peroxidase activities); and (d) a high content in iron and ascorbate can be found in some regions of the CNS, enabling generation of more ROS through the Fenton/Haber Weiss reactions.

Mitochondria also serve as high capacity Ca^{2+} sinks, which allows them to stay in tune with changes in cytosolic Ca^{2+} loads and aid in maintaining cellular Ca^{2+} homeostasis that is required for normal neuronal function (47, 83, 84). Conversely, excessive Ca^{2+} uptake into mitochondria has been shown to increase ROS production, inhibit ATP synthesis, induce mitochondrial permeability transition pore (PTP), and release small proteins that trigger the initiation of apoptosis, such as cytochrome c and apoptosis-inducing factor (AIF), from the mitochondrial intermembrane space into the cytoplasm (13, 14, 48, 95). Released cytochrome c binds apoptotic protease activating factor 1 (Apaf-1) and activates the caspase cascade (42). Such alterations in mitochondrial function have been proposed as a potential mechanism in the development and pathogenesis of AD (Fig. 1).

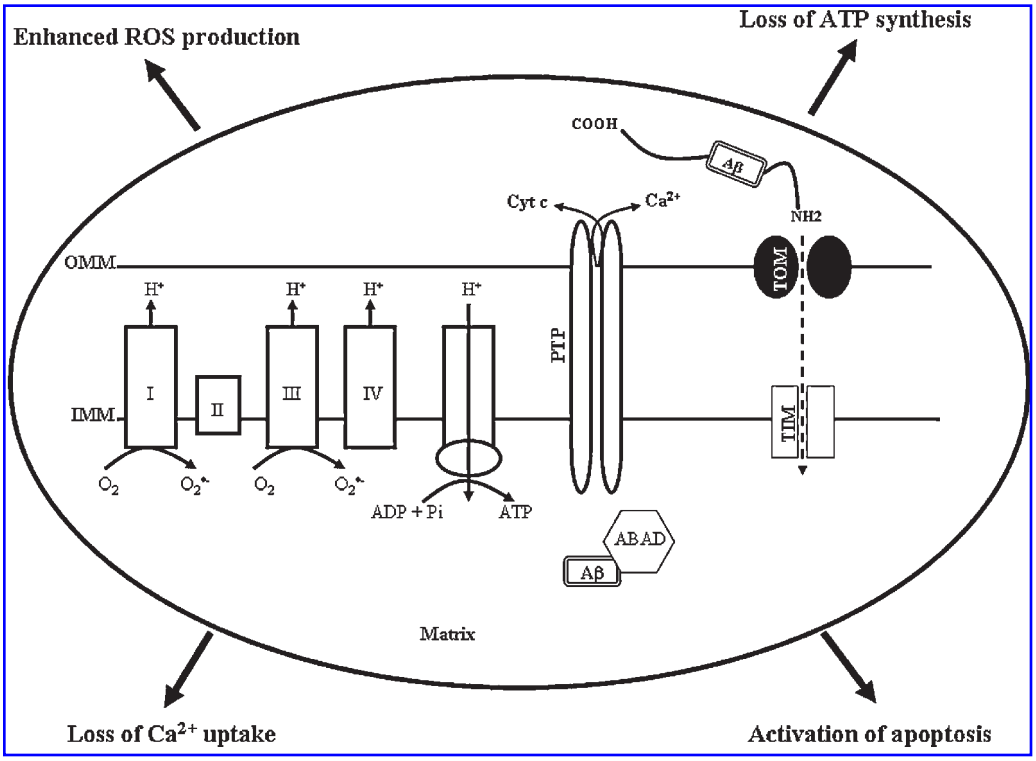

FIG. 1. Mitochondria in Alzheimer's disease. The mitochondrial permeability transition pore (PTP) is a conductance pore that spans the inner (IMM) and outer (OMM) mitochondrial membranes. Under a combination of pathophysiological conditions, such as high Ca^{2+} concentration, increased oxidative stress, low ATP, and mitochondrial depolarization, PTP opens allowing free diffusion of solutes across the membranes. The opening of the PTP ultimately results in mitochondrial swelling, mitochondrial Ca^{2+} efflux and the release of apoptogenic proteins, such as cytochrome c (Cyt c), from the intermembrane space. Amyloid β precursor protein (AβPP) has a dual leader sequence, permitting targeting to the endoplasmic reticulum or to mitochondria. Mitochondrial AβPP forms complexes with the protein importation translocases of the outer and inner membranes (TOM and TIM). However, it seems that a stretch of acidic residues prevents the total importation of AβPP that clogs the importation machinery. Consequently, the importation of respiratory chain subunits and other mitochondrial proteins is reduced. This reduced importation exacerbates the decreased activity of respiratory chain enzymes, increased reactive oxygen species (ROS) production, and impaired energy production occurring in this pathology. Amyloid-β (Aβ) has been found in mitochondria, interacting with amyloid-β-binding alcohol dehydrogenase (ABAD) and producing ROS. Additional explanations are given in the text. ADP, adenosine diphosphate; ATP, adenosine triphosphate; $O_2^{\cdot -}$, superoxide anion; Pi, inorganic phosphate.

MITOCHONDRIAL DYSFUNCTION IN ALZHEIMER'S DISEASE

Recently, Swerdlow and Khan (96) purposed the mitochondrial cascade hypothesis to explain late-onset, sporadic AD. This hypothesis states that, in the sporadic late-onset AD, mitochondrial dysfunction is the primary event that causes Aβ deposition, synaptic degeneration, and NFT formation. Indeed, there is accumulating evidence from *in vitro, in vivo*, and human studies suggesting that mitochondrial abnormalities are a common event in AD (reviewed in Ref. 61). Using in situ hybridization to mitochondrial DNA (mtDNA), immunocytochemistry of cytochrome oxidase (COX), and morphometry of electron micrographs of biopsy specimens, it was shown that mitochondrial abnormalities are intimately associated with AD (20, 43). More direct evidence for mitochondrial dysfunction in AD comes from several reports of COX deficiency in AD brains (73). Interestingly, the cellular expression of COX subunit II and IV is reduced during aging and these age-related changes are more marked in AD (74), suggesting that aging is a major risk factor for this disease. However, Cottrell and collaborators (23) observed that the distribution of amyloid plaques is anatomically distinct from the COX-deficient hippocampal pyramidal neurons, and the neurons containing NFT or apoptotic labeling were always COX-positive. The authors concluded that COX-deficient, succinate dehydrogenase-positive hippocampal neurons indicative of high mtDNA mutation load do not appear to be prone to apoptosis or to directly participate in the overproduction of tau or β-amyloid (23).

The technique of depleting cells of mtDNA has been employed to investigate the mechanisms underlying mitochondrial disorders (54). It was found that the addition of ethidium bromide to culture media resulted in a progressive depletion of intracellular mtDNA levels, leading eventually to complete and permanent loss of mtDNA. Such cells (termed ρ^0 cells) remain viable in culture due to anaerobic metabolism. Cytoplasmic hybrid cells (cybrids) were created by introducing mtDNA of interest into ρ^0 cells, and have been a central tool in unravelling effects of disease-linked mtDNA mutations. In this way, the nuclear genetic complement is held constant so that the observed effects on oxidative phosphorylation can be linked to the introduced mtDNA. It has been shown that AD cybrids replicate multiple abnormalities found in AD brain. Specifically, AD cybrids show partial reduction of COX activity that is less than that observed in AD brain and platelets, implicating mtDNA transmission of at least some of the COX deficiency (97). Associated with this COX deficiency is increased oxidative stress (52, 75, 97). Accordingly, data from our laboratory show that human teratocarcinoma cells expressing mtDNA from AD subjects display reduced COX activity, elevated ROS, and reduced ATP levels, compared with the cells expressing mtDNA from age-matched control subjects (17). These observations suggest that alterations in mtDNA may play a key role in mitochondrial dysfunction in AD.

These observations suggest that alterations in mtDNA may play a key role in mitochondrial dysfunction in AD. It has been shown that mitochondria isolated from AD platelets have a 15% decrease in COX activity, despite the fact that COX subunits are present at normal levels. Furthermore, ATP levels are diminished in AD platelets while ROS are increased (16). These results suggest that the diminished catalytic activity of COX is associated with ROS overproduction and energetic failure. Bosetti *et al*. (12) evaluated COX and F0F1-ATPase activities of isolated mitochondria from platelets of AD patients and age-matched control subjects. Compared with controls, COX activity is significantly decreased in AD platelets. In contrast, F0F1-ATPase hydrolysis activity is not significantly changed. Moreover, the ATP synthesis rate is similar in mitochondria of platelets from AD patients and controls. Using HCN-1A cells transfected with AβPP Swedish mutation, Anandatheerthavarada and colleagues (4) clearly demonstrated a time-dependent accumulation of AβPP in the mitochondria with a decline in COX activity, reduced ATP synthesis, and disruption of mitochondrial transmembrane potential. Expression of a mutant form of AβPP into PC12 cells and human embryonic kidney cells results in substantial elevation of Aβ levels and is associated with increased levels of nitric oxide and reduced ATP, finally leading to cell death (51). These findings lead to the proposal that Aβ directly disrupts mitochondrial function and may contribute to the deficiency of energy metabolism and neuronal apoptosis seen in AD. Furthermore, P19 cells stably transfected with human AβPP751 show abnormal mitochondria and decreased transmembrane potential (36).

A study by Aliev and collaborators (3) positively correlates Aβ deposition with mitochondrial abnormalities in the vascular walls of an overexpressing AβPP transgenic mice. Furthermore, a gene expression profile was carried out in an AβPP transgenic mouse model (82) to establish which genes may be critical for cellular changes in AD progression. The authors observed that genes related to mitochondrial energy metabolism and apoptosis are upregulated before and during the appearance of Aβ plaques. These results indicate that mitochondrial energy metabolism impairment, possibly by intraneuronal Aβ, could lead to an upregulation of mitochondrial genes as a compensatory response. Eckert and co-workers (26) reported that lymphocytes isolated from PS1 mutant transgenic mice show an increase in ROS production and altered Ca^{2+} regulation but no changes in mitochondrial cytochrome c content. Moreover, these mice lymphocytes show an increase in apoptotic cells as compared to nontransgenic controls, indicating that other events may be needed to trigger the mitochondrial dependent apoptotic pathway in PS1 mutations. In addition, Keil and colleagues (51) demonstrated a decrease in mitochondrial membrane potential and a reduction in ATP levels in neurons of an AβPP transgenic mouse model when compared to littermate nontransgenic mice. Those AβPP transgenic mice exhibit no plaques but an increase in Aβ levels, reinforcing the notion of a major role of intraneuronal Aβ in inducing mitochondrial dysfunction. Additionally, a recent study showed that the alterations in mitochondrial proteome and function that occur in Tg2576 mice brains appear before amyloid plaque deposition (35).

Recent data indicate that Aβ interacts with a binding protein, termed Aβ-binding alcohol dehydrogenase (ABAD), in mitochondria and directly causes mitochondrial dysfunction (56, 99). Neurons cultured from transgenic mice overexpressing AβPP and ABAD display spontaneous generation of ROS, loss of mitochondrial membrane potential, and decreased ATP, subsequent release of cytochrome c from mitochondria and induction of caspase-3-like activity followed by apoptotic cell death. Consistent with these results, a decoy peptide that blocks Aβ–ABAD interaction prevents the mitochondrial dysfunction

and apoptosis in an Aβ-rich environment *in vitro*. These results suggest that mitochondrial dysfunction and the resulting energy deficit may trigger the onset of neuronal apoptosis in AD. Moreover, Cardoso *et al*. (18) demonstrated that Aβ requires functional mitochondria to induce toxicity.

Previous studies from our laboratory also showed that Aβ1–40 and Aβ25–35 in the presence of Ca^{2+} induce the opening of PTP (62, 63). As previously referred, PTP induction, a phenomenon characterized by a sudden increase in the permeability of the inner mitochondrial membrane, plays a key role in apoptotic cell death by facilitating the release of apoptogenic factors. We observed that Aβ peptides in the presence of Ca^{2+} decrease the mitochondrial transmembrane potential and the capacity of brain mitochondria to accumulate Ca^{2+}, induce a complete uncoupling of respiration and an alteration of the ultrastructural morphology of mitochondria characterized by swelling and disruption of mitochondria cristae (62, 63). We also observed that diabetes-related mitochondrial dysfunction is exacerbated by aging and/or by the presence of Aβ, supporting the idea that diabetes and aging are risk factors for the neurodegeneration induced by these peptides (64–66). In addition, Casley and collaborators (19) reported that Aβ causes a significant reduction in states 3 and 4 of respiration and an inhibition of COX, β-ketoglutarate dehydrogenase, and pyruvate dehydrogenase activities. Data from our laboratory (76, 77) showed that Aβ25–35 and Aβ1–40 peptides decrease the activity of mitochondrial respiratory chain complexes in PC12 and NT2 cells. Another study showed that the addition of Aβ to isolated mouse brain mitochondria can directly induce cytochrome c release and mitochondrial swelling (53). Similarly, Clementi *et al*. (22) observed that exposure of isolated rat brain mitochondria to Aβ31–35 and Aβ25–35 peptides leads to release of cytochrome c, mitochondrial swelling, and a significant reduction in mitochondrial oxygen consumption. Abramov and collaborators (1) also showed that Aβ25–35 and Aβ1–42 cause a loss of mitochondrial membrane potential by activating NADPH oxidase in astrocytes. It has also been shown that Aβ25–35 induces translocation of the second-mitochondria derived activator of caspase (Smac) from mitochondria to cytosol via AP-1/Bim activation (104).

It has been demonstrated that monomeric Aβ accumulates within rat brain and muscle mitochondria (2). The authors observed four different and additive modes of action of Aβ, which were concentration-dependent: (a) an increase in mitochondrial membrane viscosity with a concomitant decrease in ATP/O ratio; (b) respiratory chain complexes inhibition; (c) an exacerbation of ROS production; and (d) cytochrome c release (2). Another study showed that intracellular Aβ1–42 selectively causes apoptosis in human neurons through p53 and Bax; the latter activates caspases by promoting release of mitochondrial cytochrome c (108).

Altogether these studies indicate a tight correlation between Aβ, mitochondrial dysfunction, and apoptosis in AD.

MITOCHONDRIAL DYSFUNCTION, OXIDATIVE STRESS, AND AUTOPHAGY

Eukaryotic cells primarily use two distinct mechanisms for large-scale degradation, the proteasome and autophagy; but only autophagy has the capacity to degrade entire organelles (reviewed in Ref. 90). The first event in autophagy is the envelopment of a mitochondrion or other targeted cytoplasmic structure in lamellae of endoplasmic reticulum. This process captures the organelles targeted for autophagy and creates double-walled structures that are isolated from the cytoplasm. These autophagosomes then acidify and acquire acid hydrolases by fusion with primary lysosomes. During this process, the inner membrane of the double-walled autophagosomes disappears, leaving behind single-membrane degradative autophagic vacuoles whose contents are ultimately digested and sometimes exocytosed (25). Autophagy occurs at basal levels in most tissues and contributes to the routine turnover of cytoplasmic components. In addition to turnover of cellular components, autophagy is involved in development, differentiation, and tissue remodeling in various organisms (reviewed in Ref. 90).

During brain aging, many mitochondria undergo enlargement and structural disorganization, while lysosomes gradually accumulate the nondegradable, polymeric lipofuscin. It is believed that this is a result of not only continuous oxidative stress, causing oxidation of mitochondrial constituents and autophagocytosed material, but also of the inherent inability of cells to completely remove oxidatively damaged structures. Although lipofuscin-loaded lysosomes continue to receive newly synthesized lysosomal enzymes, the pigment is nondegradable. Therefore, lipofuscin accumulation may greatly diminish lysosomal degradative capacity by preventing lysosomal enzymes from targeting functional autophagosomes, further limiting mitochondrial recycling. Based on findings that autophagy is diminished in lipofuscin-loaded cells and that cellular lipofuscin content positively correlates with oxidative stress and mitochondrial damage, Terman and Brunk (100) proposed the mitochondrial–lysosomal axis theory of aging, according to which mitochondrial turnover progressively declines with age, resulting in increased oxidative damage, accumulation of damaged organelles and lipofuscin, decreased ATP production, release of apoptotic factors, and, eventually, cell death (Fig. 2).

The accumulation of mutant or toxic proteins plays a major role in chronic neurodegenerative diseases (38). Morphologic evidence of autophagy has been reported in neurodegenerative diseases including AD, Parkinson disease, Huntington disease, and transmissible spongiform encephalopathies (5, 55, 80, 106, 107). Some studies suggest that autophagy activation may contribute to neurodegeneration (107). However, there is a contrasting view that autophagy may be a protective mechanism contributing to neuronal remodelling and regeneration (30, 89). Faddis and collaborators (30) assessed the role of calpain in a model of rapid, reversible dendritic injury in murine cortical cultures. The authors observed that cortical cultures briefly exposed to sublethal NMDA doses resulted in focal swellings, or varicosities, along the length of neuronal dendrites. These varicosities appeared within minutes of NMDA exposure and recovered spontaneously within 2 h after NMDA removal. Addition of the calpain inhibitors had little effect on the development of NMDA-induced dendrite injury. However, the resolution of varicosities was substantially delayed by addition of calpain inhibitors after sublethal excitotoxic exposure. Using Western blots and immunocytochemistry, the authors observed reactivity for a calpain-specific spectrin proteolytic fragment during the period of recovery from dendritic swelling, but not during its formation (30). These observations suggest that

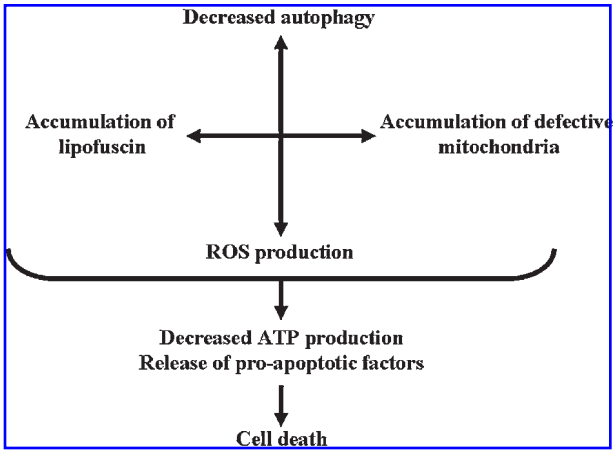

FIG. 2. The mitochondrial–lysosomal axis theory of aging. This theory states that mitochondrial turnover progressively declines with age, resulting in increased production of reactive oxygen species (ROS), accumulation of lipofuscin (biological garbage) and damaged organelles, decreased ATP production, release of apoptotic factors and, eventually, cell death.

calcium-dependent proteolysis contributes to recovery of dendritic structure. In addition, it has been reported that distinct proteolytic events, possibly involving more than one protease, regulate the initiation and subsequent elongation and stabilization of axonal neuritis (89). An early induction of macroautophagy in AD might, therefore, be expected to support the organelle and protein turnover associated with cycles of neurite degeneration and regeneration in affected neurons (30, 89), which also revert to a more immature developmental state in AD (6, 103).

Although the exact role of autophagy in AD is not fully defined, recent studies have provided some insights. Using immunogold labeling with compartmental markers and electron microscopy, Nixon and collaborators (71) identified autophagosomes and other pre-lysosomal autophagic vacuoles in AD brains particularly within neuritic processes. In dystrophic neurites, the predominant organelles are autophagosomes, multivesicular bodies, multilamellar bodies, and cathepsin-containing autophagolysosomes. Autophagy was evident in the perikarya of affected neurons, particularly in those with neurofibrillary pathology where it was associated with a relative depletion of mitochondria and other organelles. This study provides evidence that autophagy is extensively involved in the neurodegenerative/regenerative process in AD. The authors hypothesized that the accumulation of immature pre-lysosomal autophagic vacuoles in dystrophic neurites indicate that the transport of pre-lysosomal autophagic vacuoles and their maturation to lysosomes may be impaired, thereby impeding the suspected neuroprotective functions of autophagy (71). Using the PS1/AβPP mouse model of β-amyloidosis, Yu and collaborators (105) showed that neuronal autophagy is induced before extracellular deposition of Aβ. Autophagosomes and late autophagic vacuoles (AVs) accumulate markedly in dystrophic dendrites, implying an impaired maturation of AVs to lysosomes. Purified AVs contain AβPP and are highly enriched in PS1, nicastrin, and PS-dependent β-secretase activity (106). These results link β-amyloidogenic and cell survival pathways through autophagy, which is activated and is abnormal in AD. Autophagy-mediated Aβ generation has also been demonstrated by evidence that Aβ production rises when autophagy and AVs proliferation are stimulated and falls when autophagy is inhibited by blocking either of two signaling pathways converging on mTOR kinase (105). Interestingly, inclusion myositis, the only known condition in which Aβ deposits outside the nervous system (7), involves the accumulation of autophagy-related vacuoles containing AβPP, Aβ, and PS.

Although AVs accumulations are not specific to the degenerative phenomena of AD (5, 50, 107) autophagic–lysosomal pathology in the brain is considerably more widespread and robust in AD than in other adult-onset neurodegenerative diseases (70). Specifically, the extensive neuritic dystrophy (57, 87) and the characteristic gross distension of these neurites in AD are not typical in other neurodegenerative diseases lacking Aβ (10). Fibroblasts from patients with early-onset familial AD caused by mutations of presenilin-1 (PS1) abnormally accumulate AVs, especially under conditions that stimulate autophagy. Surprisingly, however, the turnover of long-lived proteins by autophagy in these fibroblasts is markedly reduced. A direct role for mutant PS1 in this defect is supported by observations that autophagy-mediated protein degradation is nearly eliminated in blastocysts from mice lacking PS1 and PS2 genes but rescued by introducing PS1 (29). Combined with evidence that PS1 and the β-secretase complex are enriched in AVs (106), these studies support that PS plays a key role in autophagy.

Recently, it has been reported that exposure of human neuroblastoma cells to hyperoxia induces the accumulation of large Aβ-containing lysosomes, which were not typical of control cells, showing a distinct localization of Aβ and lysosomal markers (110). To determine whether oxidative stress has any influence on the relationship between lysosomes and Aβ1–42, Zheng and collaborators (111) studied the effect of hyperoxia on the intracellular localization of Aβ1–42 in retinoic acid differentiated SH-SY5Y neuroblastoma cells. The authors observed that in control cells, Aβ1–42 was mainly localized to small nonlysosomal cytoplasmic granules, only occasionally Aβ1–42 was found in large lysosomal-associated membrane protein 2 positive vacuoles, devoid of the early endosomal marker rab5. These large Aβ1–42–containing lysosomes were not detectable in the presence of serum (known to suppress autophagy), while their number increased dramatically after exposure of cells to hyperoxia during 5 days (111). Altogether these results suggest a link between oxidative stress and lysosomes in AD.

As a pathogenically important pathway for Aβ generation and mediator of both cell survival and degenerative phenomena (reviewed in Ref. 85), autophagy represents a new direction for investigation into the pathogenesis and possible therapy of AD.

MITOCHONDRIA-TARGETED ANTIOXIDANTS

The knowledge that mitochondrial dysfunction has a preponderant role in AD opened a window for new therapeutic strategies aimed to preserve/ameliorate mitochondrial function.

In nearly all cases where mitochondrial dysfunction contributes to disease, a major cause of damage is ROS produced by mitochondria, either directly or as a secondary consequence of other malfunctions (8, 31). Mitochondrial oxidative damage can be decreased with clinically significant benefits by increasing the expression of mitochondrial antioxidant enzymes, or by ectopically expressing antioxidant enzymes within mitochondria (21, 88). Recently, it was shown that overexpression of catalase in mitochondria increased life span by 20% in mice, whereas overexpression of catalase in peroxisomes had no significant effect (88). Thus, antioxidant efficacy may be determined by targeted delivery to the site of ROS production.

Some beneficial effects have been reported in AD patients subjected to antioxidant supplements and diets based on CoQ10 (39, 102), vitamin E (27, 37, 67, 86), α-lipoic acid (41), and acetyl-L-carnitine (11, 45, 60). However, a major limitation in using antioxidant therapy to treat the age-related diseases, such as AD, has been the inability of investigators to enhance the antioxidant level in mitochondria (68). However, in the last years, considerable progress has been made in developing mitochondria-targeted antioxidants (*i.e.*, antioxidants that are selectively accumulated into mitochondria). Several mitochondria-targeted antioxidants have been developed by conjugating the lipophilic triphenylphosphonium (TPP^+) cation to an antioxidant moiety, such as coenzyme Q (MitCoQ) and α-tocopherol (MitoVitE) (68). This approach makes use of the potential gradient across the mitochondrial inner membrane. As a result of the proton gradient, a negative potential to 150 to 180 mV is generated across the inner membrane. Lipophilic cations may therefore accumulate 100- to 1,000-fold in mitochondria.

Data from the literature showed that the MitoVitE is taken up by mitochondria ~80-fold more than vitamin E (92). The authors observed that MitoVitE is far more effective to protect mitochondria against oxidative stress than vitamin E itself. Furthermore, it has been shown that MitoVitE is 800-fold more potent than idebenone in protecting against GSH depletion in cultured fibroblasts from patients with Friedreich ataxia and is 350–fold more potent than trolox (49). Recently, it has been shown that MitVitE mitigates ethanol-induced accumulation of intracellular oxidants and counteracts suppression of glutathione peroxidase/glutathione reductase functions, protein expression of β-glutamylcysteine synthetase and total cellular glutathione levels in cerebellar granule cells (91).

MitoQ is a promising therapeutic antioxidant that has been successfully targeted to mitochondria. Coenzyme Q (or ubiquinone) is a respiratory chain component that accepts electrons from complex I or II, to form the reduced product ubiquinol, which donates electrons to complex III. The ubiquinone pool *in vivo* exists largely in a reduced ubiquinol form, acting as an antioxidant and a mobile electron transfer. Ubiquinol has been reported to function as an antioxidant by donating a hydrogen atom from one of its hydroxyl groups to a lipid peroxyl radical, thereby decreasing lipid peroxidation within the mitochondrial inner membrane (28). MitoQ excessively accumulates in the mitochondria and reduces toxic insults from free radicals in the mitochondria. This effect ultimately leads to the protection of neurons from age-related and/or disease-related mitochondrial insults. Recently, the effects of MitoQ on mitochondria in several *in vitro* cell models were tested (9, 24, 46, 49). In cultured fibroblasts from Friedreich ataxia patients, MitoQ prevented cell death known to be caused by endogenous oxidative stress (49). In a study of PC12 cells, low concentrations of MitoQ selectively inhibited serum deprivation-induced apoptosis in PC12 cells (9). These studies suggest that MitoQ may reduce free radicals, decrease oxidative damage, and maintain mitochondrial function. Since oxidative damage is part of the known pathophysiology of AD, there is strong interest in determining whether mitochondria-targeted antioxidants decrease oxidative damage in the neurons of AD patients (81). In Phase I trials, MitoQ showed good pharmacokinetic behavior with oral dosing at 80 mg (1 mg/kg), resulting in a plasma Cmax = 33.15 ng/ml and Tmax 1 h. This formulation is now proceeding to Phase II trials in Parkinson disease and Friedreich ataxia (Antipodean Pharmaceuticals Inc., San Francisco, CA).

Recently, there was a report of a novel class of small cell-permeable peptide antioxidants that target mitochondria in a potential-independent manner (109). The structural motif of these Szeto–Schiller (SS) peptides centers on alternating aromatic residues and basic amino acids (reviewed in Ref. 98). SS-31 has a remarkable potency that can be explained by its extensive cellular uptake and selective partitioning into mitochondria. Intracellular concentrations of [3H]SS-31 were sixfold higher than extracellular concentrations. Studies using isolated mitochondria revealed that [3H]SS-31 was concentrated ~5,000-fold in the mitochondrial pellet. By concentrating in the inner mitochondrial membrane, SS-31 became localized to the site of ROS production, and protected against mitochondrial oxidative damage and against further ROS production (109). It has shown that daily injections of SS-31 to G93A SOD1 mutants, an animal model of amyotrophic lateral sclerosis, before onset of symptoms lead to a significant increase in survival and improvement of motor performance (79).

The development and improvement of mitochondria-targeted antioxidants that can protect mitochondria against oxidative stress and prevent neuronal death seems a promising therapeutic strategy in AD.

CONCLUSIONS

Many lines of evidence suggest that mitochondria have a central role in AD. Apart from providing the cell with ATP, mitochondria are also central in the regulation of cell death since they harbor several death factors that are released upon apoptotic stimuli. Alterations in mitochondrial function, increased oxidative stress, and neurons dying by apoptosis have been detected in AD patients. These findings support the idea that mitochondria may trigger the abnormal onset of neuronal degeneration and death in AD.

Until recently, autophagy has received limited attention in relation to AD, despite its importance as a mechanism for removing defective organelles and potentially toxic proteins. Defective autophagy leads to a progressive accumulation within neurons of biological "garbage," represented mainly by lipofuscin, defective mitochondria, and cytoplasmic protein aggregates. As a result of lipofuscin accumulation, the proportion of functionally effective structures declines, gradually decreasing adaptability of the biological system. In this line, it is possible

that lipofuscin deposition hampers autophagic mitochondrial turnover, promoting the accumulation of senescent mitochondria, which are deficient in ATP production but produce increased amounts of ROS. Increased oxidative stress, in turn, further enhances damage to both mitochondria and lysosomes, thus diminishing adaptability, triggering mitochondrial and lysosomal pro-apoptotic pathways, and culminating in cell death.

The knowledge that mitochondrial dysfunction has a preponderant role in AD opened a window for new therapeutic strategies aimed to preserve/ameliorate mitochondrial function. Until recently, a major limitation in developing antioxidant therapies for AD patients has been the inability to enhance antioxidant levels in mitochondria. However, a big advance occurred with the development of mitochondrial-targeted antioxidants, which preferentially enter mitochondria at several hundred-fold more than natural antioxidants. However, further studies are needed to determine whether these mitochondria-targeted antioxidants can be successfully used in age-related neurodegenerative diseases such as AD.

ABBREVIATIONS

Aβ, amyloid β peptide; ABAD, Aβ-binding alcohol dehydrogenase; AβPP, amyloid β precursor protein; AD, Alzheimer's disease; AVs, autophagic vacuoles; CNS, central nervous system; CoQ, coenzyme Q; COX, cytochrome oxidase; NFT, neurofibrillary tangle; PS, presenilin; PTP, permeability transition pore; ROS, reactive oxygen species; SP, senile plaque; TPP^+, tetraphenylphosphonium cation.

REFERENCES

1. Abramov AY, Canevari L, and Duchen MR. Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. *J Neurosci* 24: 565–575, 2004.
2. Aleardi AM, Benard G, Augereau O, Malgat M, Talbot JC, Mazat JP, Letellier T, Dachary–Prigent J, Solaini GC, and Rossignol R. Gradual alteration of mitochondrial structure and function by beta-amyloids: importance of membrane viscosity changes, energy deprivation, reactive oxygen species production, and cytochrome c release. *J Bioenerg Biomembr* 37: 207–225, 2005.
3. Aliev G, Seyidova D, Lamb BT, Obrenovich ME, Siedlak SL, Vinters HV, Friedland RP, LaManna JC, Smith MA, and Perry G. Mitochondria and vascular lesions as a central target for the development of Alzheimer's disease and Alzheimer disease-like pathology in transgenic mice. *Neurol Res* 25: 665–674, 2003.
4. Anandatheerthavarada HK, Biswas G, Robin MA, and Avadhani NG. Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. *J Cell Biol* 161: 41–54, 2003.
5. Anglade P, Vyas S, Javoy–Agid F, Herrero MT, Michel PP, Marquez J, Mouatt–Prigent A, Ruberg M, Hirsch EC, and Agid Y. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 12: 25–31, 1997.
6. Arendt T. Alzheimer's disease as a loss of differentiation control in a subset of neurons that retain immature features in the adult brain. *Neurobiol Aging* 21: 83–96, 2000.
7. Askanas V and Engel WK. Does overexpression of betaAPP in aging muscle have a pathogenic role and a relevance to Alzheimer's disease? Clues from inclusion body myositis, cultured human muscle, and transgenic mice. *Am J Pathol* 153: 1673–1677, 1998.
8. Balaban RS, Nemoto S, and Finkel T. Mitochondria, oxidants, and aging. *Cell* 120: 483–495, 2005.
9. Bedogni B, Pani G, Colavitti R, Riccio A, Borrello S, Murphy M, Smith R, Eboli ML, and Galeotti T. Redox regulation of cAMP-responsive element-binding protein and induction of manganous superoxide dismutase in nerve growth factor-dependent cell survival. *J Biol Chem* 278: 16510–9, 2003.
10. Benzing WC, Mufson EJ, and Armstrong DM. Immunocytochemical distribution of peptidergic and cholinergic fibers in the human amygdala: their depletion in Alzheimer's disease and morphologic alteration in non-demented elderly with numerous senile plaques. *Brain Res* 625: 125–138, 1993.
11. Bianchetti A, Rozzini R, and Trabucchi M. Effects of acetyl-L-carnitine in Alzheimer's disease patients unresponsive to acetylcholinesterase inhibitors. *Curr Med Res Opin* 19: 350–353, 2003.
12. Bosetti F, Brizzi F, Barogi S, Mancuso M, Siciliano G, Tendi EA, Murri L, Rapoport SI, and Solaini G. Cytochrome c oxidase and mitochondrial F1F0–ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease. *Neurobiol Aging* 23: 371–376, 2002.
13. Brustovetsky N, Brustovetsky T, Jemmerson R, and Dubinsky JM. Calcium-induced cytochrome c release from CNS mitochondria is associated with the permeability transition and rupture of the outer membrane. *J Neurochem* 80: 207–218, 2002.
14. Brustovetsky N, Brustovetsky T, Purl KJ, Capano M, Crompton M, and Dubinsky JM. Increased susceptibility of striatal mitochondria to calcium-induced permeability transition. *J Neurosci* 23: 4858–4867, 2003.
15. Budd SL and Nicholls DG. Mitochondria in the life and death of neurons. *Essays Biochem* 33: 43–52, 1998.
16. Cardoso SM, Proenca MT, Santos S, Santana I, and Oliveira CR. Cytochrome c oxidase is decreased in Alzheimer's disease platelets. *Neurobiol Aging* 25: 105–110, 2004.
17. Cardoso SM, Santana I, Swerdlow RH, and Oliveira CR. Mitochondria dysfunction of Alzheimer's disease cybrids enhances Abeta toxicity. *J Neurochem* 89: 1417–1426, 2004.
18. Cardoso SM, Santos S, Swerdlow RH, and Oliveira CR. Functional mitochondria are required for amyloid beta-mediated neurotoxicity. *FASEB J* 15: 1439–1441, 2001.
19. Casley CS, Canevari L, Land JM, Clark JB, and Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J Neurochem* 80: 91–100, 2002.
20. Castellani R, Hirai K, Aliev G, Drew KL, Nunomura A, Takeda A, Cash AD, Obrenovich ME, Perry G, and Smith MA. Role of mitochondrial dysfunction in Alzheimer's disease. *J Neurosci Res* 70: 357–360, 2002.
21. Chen Z, Siu B, Ho YS, Vincent R, Chua CC, Hamdy RC, and Chua BH. Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. *J Mol Cell Cardiol* 30: 2281–2289, 1998.
22. Clementi ME, Marini S, Coletta M, Orsini F, Giardina B, and Misiti F. Abeta(31-35) and Abeta(25-35) fragments of amyloid beta-protein induce cellular death through apoptotic signals: Role of the redox state of methionine-35. *FEBS Lett* 579: 2913–2918, 2005.
23. Cottrell DA, Borthwick GM, Johnson MA, Ince PG, and Turnbull DM. The role of cytochrome c oxidase deficient hippocampal neurones in Alzheimer's disease. *Neuropathol Appl Neurobiol* 28: 390–396, 2002.
24. Dhanasekaran A, Kotamraju S, Kalivendi SV, Matsunaga T, Shang T, Keszler A, Joseph J, and Kalyanaraman B. Supplementation of endothelial cells with mitochondria-targeted antioxidants inhibit peroxide-induced mitochondrial iron uptake, oxidative damage, and apoptosis. *J Biol Chem* 279: 37575–37587, 2004.
25. Dunn WA. Studies of the mechanisms of autophagy: maturation of the autophagic vacuole. *J Cell Biol* 110: 1935–1945, 1990.
26. Eckert A, Schindowski K, Leutner S, Luckhaus C, Touchet N, Czech C, and Muller WE. Alzheimer's disease-like alterations in peripheral cells from presenilin-1 transgenic mice. *Neurobiol Dis* 8: 331–42, 2001.

27. Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, and Breteler MM. Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA* 287: 3223–3229, 2002.
28. Ernster L, Forsmark P, and Nordenbrand K. The mode of action of lipid-soluble antioxidants in biological membranes: relationship between the effects of ubiquinol and vitamin E as inhibitors of lipid peroxidation in submitochondrial particles. Biofactors 3: 241–248, 1992.
29. Esselens C, Oorschot V, Baert V, Raemaekers T, Spittaels K, Serneels L, Zheng H, Saftig P, De Strooper B, Klumperman J, and Annaert W. Presenilin 1 mediates the turnover of telencephalin in hippocampal neurons via an autophagic degradative pathway. *J Cell Biol* 166: 1041–1054, 2004.
30. Faddis BT, Hasbani MJ, and Goldberg MP. Calpain activation contributes to dendritic remodeling after brief excitotoxic injury in vitro. *J Neurosci* 17: 951–959, 1997.
31. Finkel T. Radical medicine: treating ageing to cure disease. *Nat Rev Mol Cell Biol* 6: 971–976, 2005.
32. Fiskum G. Mitochondrial participation in ischemic and traumatic neural cell death. *J Neurotrauma* 17: 843–855, 2000.
33. Friberg H and Wieloch T. Mitochondrial permeability transition in acute neurodegeneration. *Biochimie* 84: 241–250, 2002.
34. Ghanbari HA, Ghanbari K, Harris PL Jones PK, Kubat Z, Castellani RJ, Wolozin BL, Smith MA, and Perry G. Oxidative damage in cultured human olfactory neurons from Alzheimer's disease patients. *Aging Cell* 3: 41–44, 2004.
35. Gillardon F, Rist W, Kussmaul L, Vogel J, Berg M, Danzer K, Kraut N, and Hengerer B. Proteomic and functional alterations in brain mitochondria from Tg2576 mice occur before amyloid plaque deposition. *Proteomics* 7: 605–616, 2007.
36. Grant SM, Shankar SL, Chalmers–Redman RM, Tatton WG, Szyf M, and Cuello AC. Mitochondrial abnormalities in neuroectodermal cells stably expressing human amyloid precursor protein (hAPP751). *Neuroreport* 10: 41–46, 1999.
37. Grundman M. Vitamin E and Alzheimer disease: the basis for additional clinical trials. *Am J Clin Nutr* 71: 630S–636S, 2000.
38. Grune T, Jung T, Merker K, and Davies KJ. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and "aggresomes" during oxidative stress, aging, and disease. Int J Biochem Cell Biol 36: 2519–2530, 2004.
39. Gutzmann H and Hadler D. Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease: update on a 2–year double-blind multicentre study. *J Neural Transm Suppl* 54: 301–310, 1998.
40. Halliwell B. Reactive oxygen species and the central nervous system. *J Neurochem* 59: 1609–1623, 1992.
41. Hager K, Marahrens A, Kenklies M, Riederer P, and Munch G. α-Lipoic acid as a new treatment option for Alzheimer type dementia. *Arch Gerontol Geriat* 32: 275–282, 2001.
42. Hengartner MO. The biochemistry of apoptosis. *Nature* 407: 770–776, 2000.
43. Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, and Smith MA. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci* 21: 3017–3023, 2001.
44. Honda K, Smith MA, Zhu X, Baus D, Merrick WC, Tartakoff AM, Hattier T, Harris PL, Siedlak SL, Fujioka H, Liu Q, Moreira PI, Miller FP, Nunomura A, Shimohama S, and Perry G. Ribosomal RNA in Alzheimer disease is oxidized by bound redox-active iron. *J Biol Chem* 280: 20978–20986, 2005.
45. Hudson S and Tabet N. Acetyl-L-carnitine for dementia. *Cochrane Database Syst Rev* 2: CD003158, 2003.
46. Hwang PM, Bunz F, Yu J, Rago C, Chan TA, Murphy MP, Kelso GF, Smith RA, Kinzler KW, and Vogelstein B. Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. *Nat Med* 7: 1111–1117, 2001.
47. Ichas F and Mazat JP. From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high- conductance state. *Biochim Biophys Acta* 1366: 33–50, 1998.
48. Jiang D, Sullivan PG, Sensi SL, Steward O, and Weiss JH. Zn(2+) induces permeability transition pore opening and release of pro-apoptotic peptides from neuronal mitochondria. *J Biol Chem* 276: 47524–47529, 2001.
49. Jauslin ML, Meier T, Smith RA, and Murphy MP. Mitochondria-targeted antioxidants protect Friedreich Ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. *FASEB J* 17: 1972–1974, 2003.
50. Kegel KB, Kim M, Sapp E, McIntyre C, Castano JG, Aronin N, and DiFiglia M. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. *J Neurosci* 20: 7268–7278, 2000.
51. Keil U, Bonert A, Marques CA, Scherping I, Weyermann J, Strosznajder JB, Muller–Spahn F, Haass C, Czech C, Pradier L, Muller WE, and Eckert A. Amyloid beta-induced changes in nitric oxide production and mitochondrial activity lead to apoptosis. *J Biol Chem* 279: 50310–50320, 2004.
52. Khan SM, Cassarino DS, Abramova NN, Keeney PM, Borland MK, Trimmer PA Krebs CT, Bennett JC, Parks JK, Swerdlow RH, Parker WD Jr, and Bennett JP Jr. Alzheimer's disease cybrids replicate beta-amyloid abnormalities through cell death pathways. *Ann Neurol* 48: 148–155, 2000.
53. Kim HS, Lee JH, Lee JP, Kim EM, Chang KA, Park CH, Jeong SJ, Wittendorp MC, Seo JH, Choi SH, and Suh YH. Amyloid beta peptide induces cytochrome C release from isolated mitochondria. *Neuroreport* 13: 1989–1993, 2002.
54. King MP and Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. *Science* 246: 500–503, 1989.
55. Liberski PP, Sikorska B, Bratosiewicz–Wasik J, Gajdusek DC, and Brown P. Neuronal cell death in transmissible spongiform encephalopathies (prion diseases) revisited: from apoptosis to autophagy. Int J Biochem Cell Biol 36: 2473–2490, 2004.
56. Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, Trinchese F, Liu S, Gunn–Moore F, Lue LF, Walker DG, Kuppusamy P, Zewier ZL, Arancio O, Stern D, Yan SS, and Wu H. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* 304: 448–452, 2004.
57. Masliah E, Mallory M, Deerinck T, DeTeresa R, Lamont S, Miller A, Terry RD, Carragher B, and Ellisman M. Re-evaluation of the structural organization of neuritic plaques in Alzheimer's disease. *J Neuropathol Exp Neurol* 52: 619–632, 1993.
58. Mattson MP. Pathways towards and away from Alzheimer's disease. *Nature* 430: 631–639, 2004.
59. Migliore L, Fontana I, Trippi F Colognato R, Coppede F, Tognoni G, Nucciarone B, and Siciliano G. Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. *Neurobiol Aging* 26: 567–573, 2005.
60. Montgomery SA, Thal LJ, and Amrein R. Meta-analysis of double blind randomized controlled clinical trials of acetyl-L-carnitine versus placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease. *Int Clin Psychopharmacol* 18: 61–71, 2003.
61. Moreira PI, Cardoso SM, Santos MS, and Oliveira CR. The key role of mitochondria in Alzheimer's disease. *J Alzheimers Dis* 9: 101–110, 2006.
62. Moreira PI, Santos MS, Moreno A, and Oliveira C. Amyloid beta-peptide promotes permeability transition pore in brain mitochondria. *Biosci Rep* 21: 789–800, 2001.
63. Moreira PI, Santos MS, Moreno A, Rego AC, and Oliveira C. Effect of amyloid beta-peptide on permeability transition pore: a comparative study. *J Neurosci Res* 69: 257–267, 2002.
64. Moreira PI, Santos MS, Moreno AM, Seiça R, and Oliveira CR. Increased vulnerability of brain mitochondria in diabetic (Goto–Kakizaki) rats with aging and amyloid-beta exposure. *Diabetes* 52: 1449–1456, 2003.
65. Moreira PI, Santos MS, Sena C, Nunes E, Seica R, and Oliveira CR. CoQ10 therapy attenuates amyloid beta-peptide toxicity in brain mitochondria isolated from aged diabetic rats. *Exp Neurol* 196: 112–119, 2005.
66. Moreira PI, Santos MS, Sena C, Seiça R, and Oliveira CR. Insulin protects against amyloid β-peptide toxicity in brain mitochondria of diabetic rats. *Neurobiol Dis* 18: 628–637, 2005.

67. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Wilson RS, and Scherr PA. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. *JAMA* 287: 3230–3237, 2002.
68. Murphy MP and Smith RA. Drug delivery to mitochondria: the key to mitochondrial medicine. *Adv Drug Deliv Rev* 41: 235–250, 2000.
69. Nicholls DG and Budd SL. Mitochondria and neuronal survival. *Physiol Rev* 80: 315–360, 2000.
70. Nixon RA and Cataldo AM. The lysosomal system in neuronal cell death: a review. *Ann NY Acad Sci* 679: 87–109, 1993.
71. Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, and Cuervo AM. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J Neuropathol Exp Neurol* 64: 113–122, 2005.
72. Nunomura A, Perry G, Aliev G Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, Smith MA. Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol* 60: 759–767, 2001.
73. Ojaimi J and Byrne E. Mitochondrial function and alzheimer's disease. *Biol Signals Recept* 10: 254–262, 2001.
74. Ojaimi J, Masters CL, McLean C, Opeskin K, McKelvie P, and Byrne E. Irregular distribution of cytochrome c oxidase protein subunits in aging and Alzheimer's disease. *Ann Neurol* 46: 656–660, 1999.
75. Onyango IG, Bennett JP Jr, and Tuttle JB. Endogenous oxidative stress in sporadic Alzheimer's disease neuronal cybrids reduces viability by increasing apoptosis through pro-death signaling pathways and is mimicked by oxidant exposure of control cybrids. *Neurobiol Dis* 19: 312–322, 2005.
76. Pereira C, Santos MS, and Oliveira C. Mitochondrial function impairment induced by amyloid beta-peptide on PC12 cells. *Neuroreport* 9: 1749–1755, 1998.
77. Pereira C, Santos MS, and Oliveira C. Involvement of oxidative stress on the impairment of energy metabolism induced by Abeta peptides on PC12 cells: protection by antioxidants. *Neurobiol Dis* 6: 209–219, 1999.
78. Perry G, Castellani RJ, Smith MA Harris PL, Kubat Z, Ghanbari K, Jones PK, Cordone G, Tabaton M, Wolozin B, and Ghanbari H. Oxidative damage in the olfactory system in Alzheimer's disease. *Acta Neuropathol (Berl)* 106: 552–556, 2003.
79. Petri S, Kiaei M, Damiano M, Hiller A, Wille E, Manfredi G, Calingasan NY, Szeto HH, and Beal MF. Cell-permeable peptide antioxidants as a novel therapeutic approach in a mouse model of amyotrophic lateral sclerosis. *J Neurochem* 98: 1141–1148, 2006.
80. Qin ZH, Wang Y, Kegel KB, Kazantsev A, Apostol BL, Thompson LM, Yoder J, Aronin N, and DiFiglia M. Autophagy regulates the processing of amino terminal huntingtin fragments. *Hum Mol Genet* 12: 3231–3244, 2003.
81. Reddy PH. Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease. *J Neurochem* 96: 1–13, 2006.
82. Reddy PH, McWeeney S, Park BS, Manczak M, Gutala RV, Partovi D, Jung Y, Yau V, Searles R, Mori M, and Quinn J. Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. *Hum Mol Genet* 13: 1225–1240, 2004.
83. Rizzuto R, Pinton P, Brini M, Chiesa A, Filippin L, and Pozzan T. Mitochondria as biosensors of calcium microdomains. *Cell Calcium* 26: 193–199, 1999.
84. Rizzuto R, Bernardi P, and Pozzan T. Mitochondria as all-round players of the calcium game. *J Physiol* 529: 37–47, 2000.
85. Rubinsztein DC, DiFiglia M, Heintz N, Nixon RA, Qin ZH, Ravikumar B, Stefanis L, and Tolkovsky A. Autophagy and its possible roles in nervous system diseases, damage and repair. *Autophagy* 1: 11–22, 2005.
86. Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, and Thal LJ. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N Engl J Med* 336: 1216–1222, 1997.
87. Schmidt ML, DiDario AG, Lee VM, and Trojanowski JQ. An extensive network of PHF tau-rich dystrophic neurites permeates neocortex and nearly all neuritic and diffuse amyloid plaques in Alzheimer disease. *FEBS Lett* 344: 69–73, 1994.
88. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, and Rabinovitch PS. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308: 1909–1911, 2005.
89. Shea TB, Beermann ML, and Nixon RA. Multiple proteases regulate neurite outgrowth in NB2a/dl neuroblastoma cells. *J Neurochem* 56: 842–851, 1991.
90. Shintani T and Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science* 306: 990–995, 2004.
91. Siler–Marsiglio KI, Pan Q, Paiva M, Madorsky I, Khurana NC, and Heaton MB. Mitochondrially targeted vitamin E and vitamin E mitigate ethanol-mediated effects on cerebellar granule cell antioxidant defense systems. *Brain Res* 1052: 202–211, 2005.
92. Smith RA, Porteous CM, Coulter CV, and Murphy MP. Selective targeting of an antioxidant to mitochondria. *Eur J Biochem* 263: 709–716, 1999.
93. Sullivan PG, Keller JN, Mattson MP, and Scheff SW. Traumatic brain injury alters synaptic homeostasis: implications for impaired mitochondrial and transport function. *J Neurotrauma* 15: 789–798, 1998.
94. Sullivan PG, Geiger JD, Mattson MP, and Scheff SW. Dietary supplement creatine protects against traumatic brain injury. *Ann Neurol* 48: 723–729, 2000.
95. Sullivan PG, Rabchevsky AG, Keller JN, Lovell M, Sodhi A, Hart RP, and Scheff SW. Intrinsic differences in brain and spinal cord mitochondria: implication for therapeutic interventions. *J Comp Neurol* 474: 524–534, 2004.
96. Swerdlow RH and Khan SM. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Med Hypotheses* 63: 8–20, 2004.
97. Swerdlow RH, Parks JK, Cassarino DS, Maguire DJ, Maguire RS, Bennett JP Jr, Davis RE, and Parker WD Jr. Cybrids in Alzheimer's disease: a cellular model of the disease? *Neurology* 49: 918–925, 1997.
98. Szeto HH. Mitochondria-targeted peptide antioxidants: novel neuroprotective agents. *AAPS J* 8: E521–31, 2006.
99. Takuma K, Yao J, Huang J, Xu H, Chen X, Luddy J, Trillat AC, Stern DM, Arancio O, and Yan SS. ABAD enhances Abeta-induced cell stress via mitochondrial dysfunction. *FASEB J* 19: 597–598, 2005.
100. Terman A and Brunk UT. Oxidative stress, accumulation of biological 'garbage', and aging. *Antioxid Redox Signal* 8: 197–204, 2006.
101. Wieloch T. Mitochondrial involvement in acute neurodegeneration. *IUBMB Life* 52: 247–254, 2001.
102. Weyer G, Babej–Dolle RM, Hadler D, Hofmann S, and Herrmann WM. A controlled study of 2 doses of idebenone in the treatment of Alzheimer's disease. *Neuropsychobiology* 36: 73–82, 1997.
103. Yang Y, Mufson EJ, and Herrup K. Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease. *J Neurosci* 23: 2557–2563, 2003.
104. Yin KJ, Lee JM, Chen SD, Xu J, and Hsu CY. Amyloid-beta induces Smac release via AP-1/Bim activation in cerebral endothelial cells. Amyloid-beta induces Smac release via AP-1/Bim activation in cerebral endothelial cells. *J Neurosci* 22: 9764–9770, 2002.
105. Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, Mohan PS, Mercken M, Farmery MR, Tjernberg LO, Jiang Y, Duff K, Uchiyama Y, Naslund J, Mathews PM, Cataldo AM, and Nixon RA. Macroautophagy–a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. *J Cell Biol* 171: 87–98, 2005.
106. Yu WH, Kumar A, Peterhoff C, Shapiro Kulnane L, Uchiyama Y, Lamb BT, Cuervo AM, and Nixon RA. Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localiza-

tion in Alzheimer's disease. Int J Biochem Cell Biol 36: 2531–2540, 2004.
107. Yue Z, Horton A, Bravin M, DeJager PL, Selimi F, and Heintz N. A novel protein complex linking the δ2 glutamate receptor and autophagy: implications for neurodegeneration in lurcher mice. Neuron 35: 921–933, 2002.
108. Zhang Y, McLaughlin R, Goodyer C, and LeBlanc A. Selective cytotoxicity of intracellular amyloid beta peptide1–42 through p53 and Bax in cultured primary human neurons. *J Cell Biol* 156: 519–529, 2002.
109. Zhao K, Zhao GM, Wu D, Soong Y, Birk AV, Schiller PW, and Szeto HH. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J Biol Chem* 279: 34682–34690, 2004.
110. Zheng L, Roberg K, Jerhammar F, Marcusson J, and Terman A. Oxidative stress induces intralysosomal accumulation of Alzheimer amyloid beta-protein in cultured neuroblastoma cells. *Ann NY Acad Sci* 1067: 248–251, 2006.
111. Zheng L, Roberg K, Jerhammar F, Marcusson J, and Terman A. Autophagy of amyloid beta-protein in differentiated neuroblastoma cells exposed to oxidative stress. *Neurosci Lett* 394: 184–189, 2006.

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2. J.S. Cruz, C. Kushmerick, D.C. Moreira-Lobo, F.A. Oliveira. 2012. Thiamine deficiency in vitro accelerates A-type potassium current inactivation in cerebellar granule neurons. *Neuroscience* **221**, 108-114. [CrossRef]
3. Inês Marques-Aleixo, Paulo J. Oliveira, Paula I. Moreira, José Magalhães, António Ascensão. 2012. Physical exercise as a possible strategy for brain protection: Evidence from mitochondrial-mediated mechanisms. *Progress in Neurobiology* . [CrossRef]
4. Gabriela K. Ferreira, Giselli Scaini, Milena Carvalho-Silva, Lara M. Gomes, Lislaine S. Borges, Júlia S. Vieira, Larissa S. Constantino, Gustavo C. Ferreira, Patrícia F. Schuck, Emilio L. Streck. 2012. Effect of l-Tyrosine In Vitro and In Vivo on Energy Metabolism Parameters in Brain and Liver of Young Rats. *Neurotoxicity Research* . [CrossRef]
5. Paula Moreira, Catarina OliveiraMitochondrial Abnormalities and Oxidative Stress in Alzheimer's Disease **30**, 391-414. [CrossRef]
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7. Isidro Ferrer. 2012. Defining Alzheimer as a common age-related neurodegenerative process not inevitably leading to dementia. *Progress in Neurobiology* **97**:1, 38-51. [CrossRef]
8. Ester Aso, Selene Lomoio, Irene López-González, Laura Joda, Margarita Carmona, Núria Fernández-Yagüe, Jesús Moreno, Salvador Juvés, Aurora Pujol, Reinald Pamplona, Manuel Portero-Otin, Virginia Martín, Mario Díaz, Isidro Ferrer. 2012. Amyloid Generation and Dysfunctional Immunoproteasome Activation with Disease Progression in Animal Model of Familial Alzheimer's Disease. *Brain Pathology* no-no. [CrossRef]
9. Ana I. Duarte, Paula I. Moreira, Catarina R. Oliveira. 2012. Insulin in Central Nervous System: More than Just a Peripheral Hormone. *Journal of Aging Research* **2012**, 1-21. [CrossRef]
10. Sónia C. Correia, Renato X. Santos, Cristina Carvalho, Susana Cardoso, Emanuel Candeias, Maria S. Santos, Catarina R. Oliveira, Paula I. Moreira. 2012. Insulin signaling, glucose metabolism and mitochondria: Major players in Alzheimer's disease and diabetes interrelation. *Brain Research* . [CrossRef]
11. Anjali Sharma, Ghareb M. Soliman, Noura Al-Hajaj, Rishi Sharma, Dusica Maysinger, Ashok Kakkar. 2011. Design and Evaluation of Multifunctional Nanocarriers for Selective Delivery of Coenzyme Q10 to Mitochondria. *Biomacromolecules* 111216122336008. [CrossRef]
12. Gabriela K. Ferreira, Gislaine T. Rezin, Mariane R. Cardoso, Cinara L. Gonçalves, Lislaine S. Borges, Júlia S. Vieira, Lara M. Gomes, Alexandra I. Zugno, João Quevedo, Emilio L. Streck. 2011. Brain energy metabolism is increased by chronic administration of bupropion. *Acta Neuropsychiatrica* no-no. [CrossRef]
13. Michael Zick, Andreas S. ReichertMitochondria 87-111. [CrossRef]
14. Pollyana Feldhaus, Daiane B. Fraga, Fernando V. Ghedim, Renata D. Luca, Thiago D. Bruna, Matheus Heluany, Maria Paula Matos, Gabriela K. Ferreira, Isabela C. Jeremias, Claudia Heluany, Emilio L. Streck, Alexandra I. Zugno. 2011. Evaluation of respiratory chain activity in lymphocytes of patients with Alzheimer disease. *Metabolic Brain Disease* . [CrossRef]
15. Arne Ittner, Yazi D. Ke, Janet van Eersel, Amadeus Gladbach, Jürgen Götz, Lars M. Ittner. 2011. Brief update on different roles of tau in neurodegeneration. *IUBMB Life* **63**:7, 495-502. [CrossRef]
16. Giselli Scaini, Débora D. Maggi, Bruna T. De-Nês, Cinara L. Gonçalves, Gabriela K. Ferreira, Brena P. Teodorak, Gisele D. Bez, Gustavo C. Ferreira, Patricia F. Schuck, João Quevedo, Emilio L. Streck. 2011. Activity of mitochondrial respiratory chain is increased by chronic administration of antidepressants. *Acta Neuropsychiatrica* **23**:3, 112-118. [CrossRef]
17. Renato X. Santos, Sónia C. Correia, Susana Cardoso, Cristina Carvalho, Maria S. Santos, Paula I. Moreira. 2011. Effects of rapamycin and TOR on aging and memory: implications for Alzheimer's disease. *Journal of Neurochemistry* **117**:6, 927-936. [CrossRef]
18. Sónia C. Correia, Renato X. Santos, George Perry, Xiongwei Zhu, Paula I. Moreira, Mark A. Smith. 2011. Insulin-resistant brain state: The culprit in sporadic Alzheimer's disease?. *Ageing Research Reviews* **10**:2, 264-273. [CrossRef]

19. Xiaoxia Tang, Lawrence M. Sayre, Gregory P. Tochtrop. 2011. A mass spectrometric analysis of 4-hydroxy-2-(E)-nonenal modificationof cytochrome c. *Journal of Mass Spectrometry* **46**:3, 290-297. [CrossRef]
20. Anne Eckert, Karen Schmitt, Jürgen Götz. 2011. Mitochondrial dysfunction - the beginning of the end in Alzheimer's disease? Separate and synergistic modes of tau and amyloid-# toxicity. *Alzheimer's Research & Therapy* **3**:2, 15. [CrossRef]
21. Lucia Pagani, Anne Eckert. 2011. Amyloid-Beta Interaction with Mitochondria. *International Journal of Alzheimer's Disease* **2011**, 1-12. [CrossRef]
22. Dusica Maysinger, Sebastien Boridy, Eliza HutterSubcellular Fate of Nanodelivery Systems 93-121. [CrossRef]
23. Federica Valsecchi, Werner J.H. Koopman, Ganesh R. Manjeri, Richard J. Rodenburg, Jan A.M. Smeitink, Peter H.G.M. Willems. 2010. Complex I disorders: Causes, mechanisms, and development of treatment strategies at the cellular level. *Developmental Disabilities Research Reviews* **16**:2, 175-182. [CrossRef]
24. Anne Eckert, Kathrin L. Schulz, Virginie Rhein, Jürgen Götz. 2010. Convergence of Amyloid-# and Tau Pathologies on Mitochondria In Vivo. *Molecular Neurobiology* **41**:2-3, 107-114. [CrossRef]
25. Walter E. Müller, Anne Eckert, Christopher Kurz, Gunter Peter Eckert, Kristina Leuner. 2010. Mitochondrial Dysfunction: Common Final Pathway in Brain Aging and Alzheimer's Disease—Therapeutic Aspects. *Molecular Neurobiology* **41**:2-3, 159-171. [CrossRef]
26. Yun-An Lim, Virginie Rhein, Ginette Baysang, Fides Meier, Anne Poljak, Mark J. Raftery, Michael Guilhaus, Lars M. Ittner, Anne Eckert, Jürgen Götz. 2010. A# and human amylin share a common toxicity pathway via mitochondrial dysfunction. *PROTEOMICS* **10**:8, 1621-1633. [CrossRef]
27. Anna Martínez, Manuel Portero-Otin, Reinald Pamplona, Isidre Ferrer. 2010. Protein Targets of Oxidative Damage in Human Neurodegenerative Diseases with Abnormal Protein Aggregates. *Brain Pathology* **20**:2, 281-297. [CrossRef]
28. Sónia C. Correia, Paula I. Moreira. 2010. Hypoxia-inducible factor 1: a new hope to counteract neurodegeneration?. *Journal of Neurochemistry* **112**:1, 1-12. [CrossRef]
29. Paula I. Moreira, Xiongwei Zhu, Xinglong Wang, Hyoung-gon Lee, Akihiko Nunomura, Robert B. Petersen, George Perry, Mark A. Smith. 2010. Mitochondria: A therapeutic target in neurodegeneration. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1802**:1, 212-220. [CrossRef]
30. Paula I. Moreira, Cristina Carvalho, Xiongwei Zhu, Mark A. Smith, George Perry. 2010. Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1802**:1, 2-10. [CrossRef]
31. Giorgio Lenaz, Paola StrocchiReactive Oxygen Species in the Induction of Toxicity . [CrossRef]
32. Jin-Tai Yu, Raymond Chuen-Chung Chang, Lan Tan. 2009. Calcium dysregulation in Alzheimer's disease: From mechanisms to therapeutic opportunities. *Progress in Neurobiology* **89**:3, 240-255. [CrossRef]
33. S. Hauptmann, I. Scherping, S. Dröse, U. Brandt, K.L. Schulz, M. Jendrach, K. Leuner, A. Eckert, W.E. Müller. 2009. Mitochondrial dysfunction: An early event in Alzheimer pathology accumulates with age in AD transgenic mice. *Neurobiology of Aging* **30**:10, 1574-1586. [CrossRef]
34. Isidre Ferrer. 2009. Altered mitochondria, energy metabolism, voltage-dependent anion channel, and lipid rafts converge to exhaust neurons in Alzheimer's disease. *Journal of Bioenergetics and Biomembranes* **41**:5, 425-431. [CrossRef]
35. Cristina Carvalho, Sónia C. Correia, Renato X. Santos, Susana Cardoso, Paula I. Moreira, Timothy A. Clark, Xiongwei Zhu, Mark A. Smith, George Perry. 2009. Role of mitochondrial-mediated signaling pathways in Alzheimer disease and hypoxia. *Journal of Bioenergetics and Biomembranes* **41**:5, 433-440. [CrossRef]
36. You-Jun Fu, Shuling Xiong, Mark A. Lovell, Bert C. Lynn. 2009. Quantitative Proteomic Analysis of Mitochondria in Aging PS-1 Transgenic Mice. *Cellular and Molecular Neurobiology* **29**:5, 649-664. [CrossRef]
37. Roland Jakob-Roetne, Helmut Jacobsen. 2009. Die Alzheimer-Demenz: von der Pathologie zu therapeutischen Ansätzen. *Angewandte Chemie* **121**:17, 3074-3105. [CrossRef]
38. Roland Jakob-Roetne, Helmut Jacobsen. 2009. Alzheimer's Disease: From Pathology to Therapeutic Approaches. *Angewandte Chemie International Edition* **48**:17, 3030-3059. [CrossRef]
39. Young-Sook Choi, Sang-Ho Kim. 2009. Mitochondrial Dysfunction and Apoptosis Related Gene Expression in A#25-35-Treated Human Neuroblastoma Cell Line, SK-N-SH. *Journal of the Korean Geriatrics Society* **13**:3, 142. [CrossRef]
40. M BOLOGNESI, A CAVALLI, C MELCHIORRE. 2009. Memoquin: A Multi-Target–Directed Ligand as an Innovative Therapeutic Opportunity for Alzheimer's Disease. *Neurotherapeutics* **6**:1, 152-162. [CrossRef]

41. Gislaine T. Rezin, Mariane R. Cardoso, Cinara L. Gonçalves, Giselli Scaini, Daiane B. Fraga, Rafael E. Riegel, Clarissa M. Comim, João Quevedo, Emilio L. Streck. 2008. Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. *Neurochemistry International* **53**:6-8, 395-400. [CrossRef]
42. Paula I. Moreira, Akihiko Nunomura, Masao Nakamura, Atsushi Takeda, Justin C. Shenk, Gjumrakch Aliev, Mark A. Smith, George Perry. 2008. Nucleic acid oxidation in Alzheimer disease. *Free Radical Biology and Medicine* **44**:8, 1493-1505. [CrossRef]
43. P. Hemachandra Reddy, M. Flint Beal. 2008. Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. *Trends in Molecular Medicine* **14**:2, 45-53. [CrossRef]