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Review article

Alzheimer's disease: the role of mitochondrial dysfunction and potential new therapies

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Alzheimer's disease (AD) is characterized by neuronal loss and gradual cognitive impairment. AD is the leading cause of dementia worldwide and the incidence is increasing rapidly, with diagnoses expected to triple by the year 2050. Impaired cholinergic transmission is a major role player in the rapid deterioration associated with AD, primarily as a result of increased acetylcholinesterase (AChE) in the AD brain, responsible for reducing the amount of acetylcholine (ACh). Current drug therapies, known as AChE inhibitors (AChEIs), target this heightened level of AChE in an attempt to slow disease progression. AChEIs have only showed success in the treatment of mild to moderate AD symptoms, with the glutamate inhibitor memantine being the most common drug prescribed for the management of severe AD. As these drugs simply delay the onset of symptoms, the development of new therapies is key. As neurons are highly energy-demanding cells, they rely heavily on the functions of mitochondria, and any dysfunction affecting respiratory processes can be devastating and lead to the neuronal death characteristic of AD. Dysfunction in fission and fusion processes of mitochondria have been observed in early AD and are heavily involved in AD pathogenesis. Beta-amyloid (Aβ) is a neurotoxic protein formed in the AD brain as a result of inappropriate secretase activity and is one of the major hallmarks of the disease. Aβ has recently been discovered in the membranes of mitochondria, disabling many basic respiratory functions. Ongoing research is largely targeted at protecting mitochondria from damage caused by factors such as Aβ and oxidative stress. Antioxidants have been meticulously studied, and several generic antioxidants such as α-tocopherol have been found to significantly slow the rate of cognitive decline in both mild to moderate and severe AD. MitoQ is a mitochondria specific antioxidant which is able to enter mitochondria in an almost thousand fold greater concentration than is achieved by generic antioxidants. This enables protection against potentially devastating factors for mitochondria, such as lipid peroxidation, oxidative stress and Aβ neurotoxicity. This review further discusses mitochondrial therapies as well as other new treatments for AD.

Key words: β-amyloid, acetylcholine, mitochondria, MitoQ, antioxidants, reactive oxygen species (ROS)

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Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide. Dementia patients totalled 24.2 million in 2005 and 4.2 million cases arose each year from 2005 to 2011, with 70% of these cases being a result of AD (Christiane, Brayne and Mayeux, 2011). AD predominantly affects more women than men, and the number of people dying as a result of the disease increased by 55.7 people per hundred thousand in the USA from 2000 to 2010 (Centers for Disease Control and Prevention, NCHS, 2008). Frequently, the cause of death of AD sufferers is a secondary condition such as pneumonia

or ischaemic heart disease which can be exacerbated by many of the symptoms of AD (Brunnstrom and Englund, 2009). The number of people developing AD is expected to triple by the year 2050 (Mohammadi-Khanaposhtani *et al.*, 2015), when it is estimated that 4.1% of the population will be over 80 years of age (Department of Economic and Social Affairs, 2001). In developed countries, 1 in 10 people over the age of 65 are affected by dementia of some form, with the frequency of AD almost doubling within this specific population every 5 years (Qiu, Kivipelto and von Strauss, 2009). Worldwide, the cost of medical care for dementia sufferers totals approximately 604 billion US$ (Fargo *et al.*, 2014), with the annual cost of AD per patient ranging from between US$42 000 and US$56 000 in the USA (Hurd *et al.*, 2013). Clearly AD is both widespread and costly.

AD is a progressive, age-associated neurodegenerative disease which is characterized by neuronal loss and accompanying cognitive impairment (Walsh and Selkoe, 2007; Bonda *et al.*, 2010), with symptoms ranging from 'preclinical' (Sperling *et al.*, 2011) to severe. Early symptoms of AD may first be mistaken by friends and family members as normal signs of ageing, however as the disease progresses symptoms worsen rapidly, though the rate at which this happens differs between individuals. Later stages of the disease result in the sufferer being unable to perform everyday tasks such as carrying out basic hygiene routines, or being able to bathe or eat independently (Leifer, 2009). AD commonly goes undiagnosed until it reaches these more debilitating stages. The mini mental state examination is used to assess the level of cognitive impairment that a dementia patient may be experiencing via a series of exercises, such as memorizing a list of objects or correctly answering time-orientation questions (NHS-UK, 2015). Further tests such as blood tests and MRI or CT scans may then be taken, characteristically showing diminished brain tissue as a result of neuronal loss, forming a definitive diagnosis (Cullen *et al.*, 2007).

The most popular theory regarding AD onset is the role of Aβ, a fundamental component of extracellular plaques that accumulate in the brains of AD sufferers via amyloidogenesis (Picone *et al.*, 2014). This process is known to be a leading cause of the neuronal loss that can be observed in AD and is a recognized hallmark of the disease (Bonda *et al.*, 2010; Das, Murray and Belfort, 2015). Similarly, microtubule associated tau proteins become hyperphosphorylated and form neurofibrillary tangles which is also recognized as a hallmark of the disease (Bonda *et al.*, 2010; Annamalai *et al.*, 2015).

It is thought that as well as these known hallmarks, mitochondrial malfunctions play a distinct role in AD pathogenesis (Obulesu and Lakshmi, 2014). The processes of fission and fusion are vital in mitochondrial dynamics in order to maintain a balance of the morphology, number, distribution, and function of these organelles within cells (Wang *et al.*, 2009). When these processes become unbalanced, mitochondria are unable to adequately carry out their functions making them vulnerable to consequences such as oxidative stress which can lead to the neurodegeneration typically seen in AD (Bonda *et al.*, 2010).

This review will outline the pathophysiology of AD and the current medications used, and primarily focus on newer treatment strategies such as therapies targeted at mitochondrial dysfunction in neurons. It will explain the possible benefits of these newer treatment techniques, currently undergoing clinical testing, and demonstrate the difficulties associated with finding successful AD therapies.

Pathophysiology of AD and currently available medications

Pathophysiology of AD

Amyloid precursor protein (APP) is a type I transmembrane protein which is synthesized in the endoplasmic reticulum (O'Brien and Wong, 2011) and found in the neuronal cell membrane, with a large extracellular N-terminus and a shorter intracellular C-terminus. APP must be cleaved into smaller fragments by proteases in order to be functional. In healthy brains, a protease known as α-secretase carries out the first cleavage followed by a secondary cleavage from γ-secretase, giving rise to the nonamyloidgenic pathway. This forms the alpha C-terminal fragment and also causes APP to release its extracellular domain, known as APPsα (Obregon *et al.*, 2012), which are thought to be beneficial to neurons (Zhang *et al.*, 2011). In AD, APP is formed incorrectly due to an excess of β-secretase. When APP is first cleaved by β-secretase as opposed to α-secretase, C-terminal fragment β-CTF along with the soluble N-terminal fragment APPsβ are generated. γ-secretase further processes the β-CTF fragment, forming Aβ fragments of varying lengths; Aβ40 and Aβ42 being the primary toxic species found in AD brains (Obregon *et al.*, 2012). This is known as the amyloidgenic pathway (Obregon *et al.*, 2012; Picone *et al.*, 2014) (Fig. 1). As more Aβ40 and Aβ42 are released, the Aβ oligomers increase in size and become insoluble and increasingly toxic to neurons, forming the aforementioned Aβ plaques (Hayden and Teplow, 2013).

In the brains of healthy individuals, tau stabilizes components critical to the internal transport system of neurons. Tau attaches to microtubules along the length of the neuron, allowing nutrients and other metabolic substances to be transported throughout the cell (Guzman-Martinez, Farias and Maccioni, 2013). In AD brains, tau is modified, causing it to separate from the microtubules therefore resulting in their degradation. Intracellular tangles formed by tau protein hyperphosphorylation disable the transport system and inactivate the neuron. Neurons are unable to regenerate, therefore as these processes continue neurons begin to disconnect from each other and eventually die, leading to memory loss, cognitive decline and other symptoms associated with AD as the brain tissue gradually shrinks and loses function.

In AD patients, cholinergic pathways become compromised in the basal forebrain and cerebral cortex (Herholz, 2008), primarily due to an excess amount of acetylcholinesterase (AChE), leading to a decrease in acetylcholine (ACh) levels (Zhou *et al.*, 2015). These cholinergic deficits are

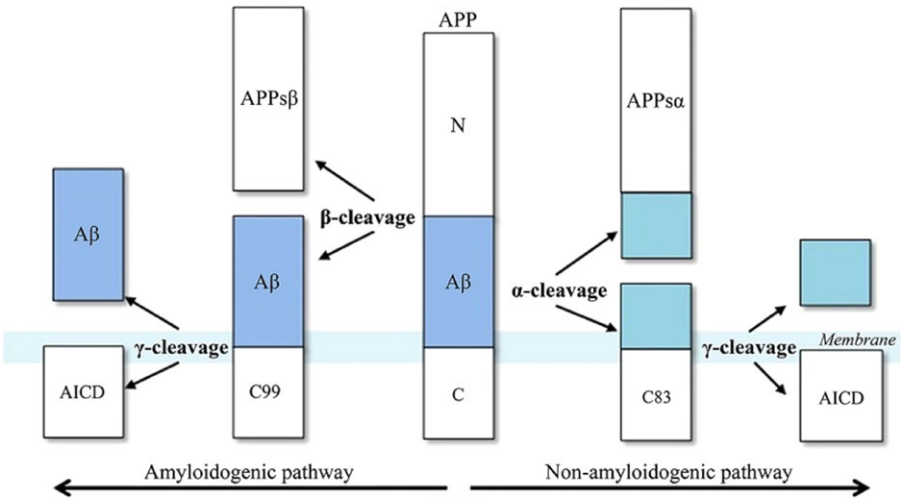

Figure 1. Nonamyloidgenic and amyloidgenic pathways originate from different APP processing. The nonamyloidgenic pathway sees cleavage of α-secretase produce the healthy APPsα fragment, with further cleavage of γ-secretase giving rise to no Aβ generation. In AD, β-secretase forms the initial cleavage producing the APPsβ fragment, with γ-secretase combining to produce Aβ plaques in the amyloidgenic pathway. (Reproduced from Menting and Claassen 2014, open access under the Creative Commons Attribution License CC-BY.)

thought by many to be a major factor in AD (Herholz, 2008; Biswas *et al.*, 2015; Zhou *et al.*, 2015). Evidence suggests that AChE can also interact with Aβ and increase the number and toxicity of Aβ plaques via interaction with AChEs peripheral binding site (Mantoani *et al.*, 2016).

Currently available medications to target neurotransmitters

ACh has been found to be greatly reduced in sufferers of AD compared to healthy controls (Ankarcrona, Mangialasche and Winblad, 2010; Zhou *et al.*, 2015). Drugs currently available on the UK National Health Service work primarily by reducing AChE levels and therefore restoring levels of cholinergic transmission in the brain. Drugs can also be prescribed which target glutamatergic transmission, as both glutamatergic and cholinergic transmission are impaired in the brains of AD sufferers (Cacabelos, 2007). There are four main drugs currently in use for the relief of symptoms of AD: donepezil, rivastigmine, galantamine and, usually only prescribed for severe AD, memantine. Donepezil is the most commonly prescribed drug for AD in more than 50 countries. It is a highly selective AChE inhibitor (AChEI) with a response rate of 40–58%, improving behaviour, cognition and quality of life in both moderate and severe AD (Sadowsky *et al.*, 2014). Rivastigmine is another commonly prescribed AChEI. It is often a treatment of preference as it also inhibits butylcholinesterase, increased in those with AD and causing an imbalance with decreased levels of AChE (Mushtag *et al.*, 2014), and it can be administered both orally and via transdermal patch (Cacabelos, 2007; Sasaki and Horie, 2014; Farlow *et al.*, 2015), which can be beneficial for some patients if they display more violent and restless symptoms. Galantamine is a newer drug, and is an established AChEI which is also an allosteric modulator affecting nicotinic ACh receptors, alleviating both behavioural and psychiatric symptoms of mild to moderate AD (Cacabelos, 2007; Farokhnia *et al.*, 2014). These three drugs are medications predominantly prescribed for mild to moderate AD, effective by restoring cholinergic pathways and delaying symptom progression.

The majority of drug therapies for AD focus on treating mild to moderate AD symptoms, with only a minority being targeted at severe AD. Memantine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist (Zhu *et al.*, 2013), is the leading drug in the treatment for severe AD along with donepezil and often the two are used in conjunction (Wenk, Parsons and Danysz, 2006). Memantine works primarily by blocking excess levels of glutamate, therefore preventing glutamatergic toxicity which can be fatal for neurons in AD brains. This is achieved without affecting normal glutamatergic transmission (Wenk, Parsons and Danysz, 2006). Memantine, though not as widely used as AChEIs, does show potential neuroprotective properties such as decreasing tau protein hyperphosphorylation and consequentially inhibiting neurofibrillary tangle formation and Aβ deposition, as well as reducing the amount of damage caused to neural cells by aiding the reduction of abnormal synaptic signals (Cacabelos, 2007).

Role of mitochondria in disease pathogenesis

Mitochondrial dynamics in neuronal cells: Fission, fusion and function

Mitochondria are constantly dividing and fusing within cells depending on environmental demands (Chan, 2006). Neurons are highly demanding cells with regards to mitochondria and require

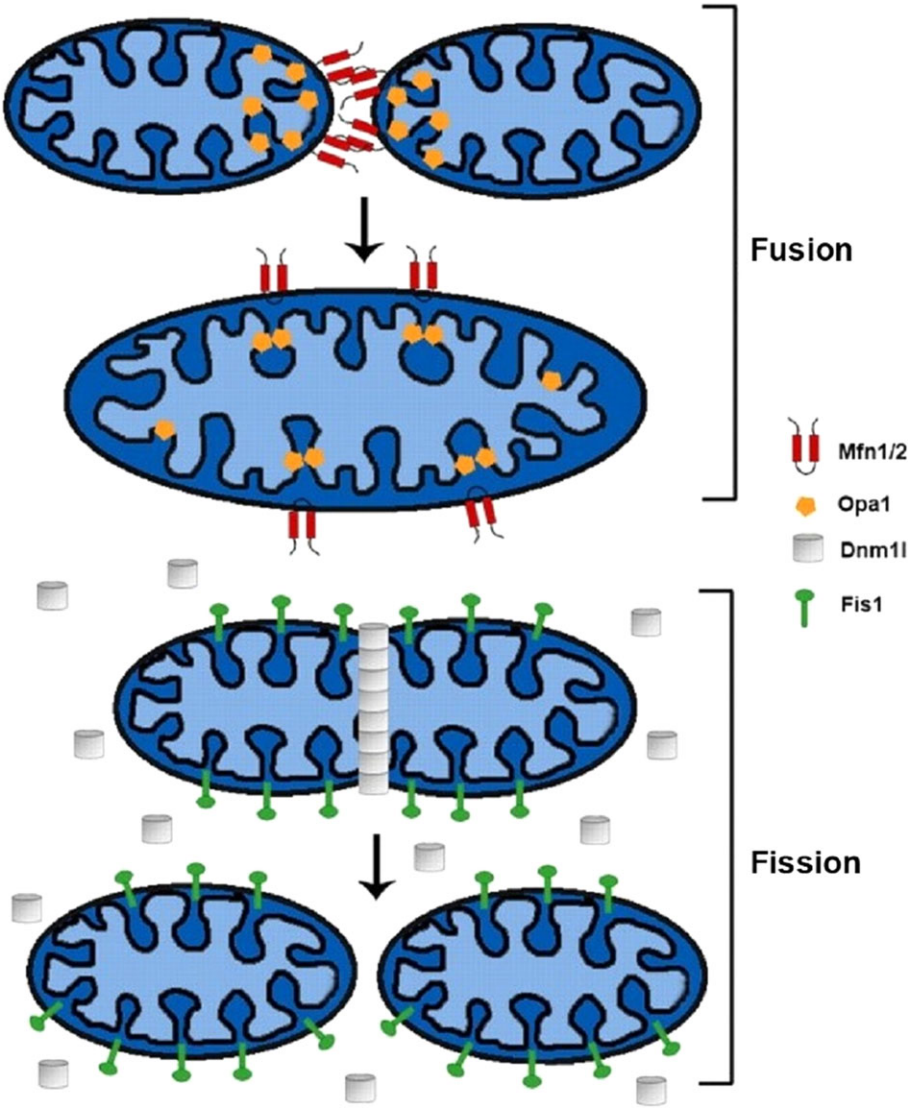

Figure 2. Schematic representation of mitochondrial fission and fusion events, regulated by the proteins: Mfn 1 and 2, Opa1, Dnm1L and Fis1. (Adapted with permission from the Company of Biologists Ltd., Mandemakers, Morais and De Strooper 2007).

large amounts of energy. Mitochondria provide the majority of their energy to the cell through oxidative phosphorylation during the TCA cycle (Knott and Bossy-Wetzel, 2008; Bonda *et al.*, 2010), and provide energy for many ATP-dependent neuronal processes such as synaptic transmission, vesicle release, ion channel and receptor-related processes and the reuptake and recycling of neurotransmitters (Knott and Bossy-Wetzel, 2008).

Fission and fusion are the two main processes by which the mitochondria remain in synchronization with the energy demands of cells (Fig. 2). These processes also allow the spread of mitochondrial DNA (mtDNA) and metabolites during fusion processes (Santos *et al.*, 2010), and keep the amount of defective mitochondria in the cell at a low level during fission (Bonda *et al.*, 2010). Both processes are largely mediated by guanosine triphosphatase (GTPase) enzymes. Fission, the process of two mitochondria arising from one mitochondrial division, largely relies on two proteins: the GTPase dynamin like protein 1 (DLP-1, or DNM1L), a cytosolic protein believed to be recruited to the outer mitochondrial membrane when required; and the small protein Fis1 (Bonda *et al.*, 2010; Santos *et al.*, 2010). Fis1 is an outer membrane protein, and is believed to be a DLP-1 receptor and involved in DLP-1 recruitment; however, the exact mechanism is still unknown (Knott and Bossy-Wetzel, 2008; Santos *et al.*, 2010). Fusion of mitochondria is regulated by the large GTPases mitofusin 1 (Mfn1), mitofusin 2 (Mfn2) and optic atrophy protein 1 (OPA1) (Bonda *et al.*, 2010; Santos *et al.*, 2010). Mfn1 and Mfn2 are transmembrane proteins which span the outer mitochondrial membrane and are involved in connecting the outer

membranes of nearby mitochondria to each other. However, the inner membrane must also fuse to allow the intracellular contents to merge together, which is where OPA1 is involved (Santos *et al.*, 2010). OPA1 is an inner membrane protein which faces the intermembrane space, and requires Mfn1, but not necessarily Mfn2, to mediate the process of inner mitochondrial membrane (IMM) fusion (Santos *et al.*, 2010).

As well as the quantity of mitochondria in a cell at any one time, the position within the cell is crucial. Within cells, mitochondria are mobile via the cytoskeleton tracks (Lacker, 2013). Axonal mitochondria motility is regulated by intracellular and mitochondrial matrix Ca^{2+} concentration; the number of moving mitochondria in the axon is mediated primarily through neuronal activity (Obashi and Okabe, 2013). Morphologically, abnormal fission and fusion mitochondria, elongated and short round mitochondria, respectively, also cause distribution changes within the cell. These processes, as well as the cytoskeleton, play pivotal roles in maintaining cell integrity and therefore any changes in these processes can have drastic consequences, such as the neurodegeneration observed in AD (Bonda *et al.*, 2010).

Mitochondrial dysfunction in AD

Aβ in mitochondria

Aβ has recently been found in mitochondria (Picone *et al.*, 2014), accumulating in post-mortem AD brains, AD brains of living patients and the brains of transgenic AD mice (Ankarcrona, Mangialasche and Winblad, 2010). Aβ is present in mitochondria prior to amyloid plaque formation, suggesting that mitochondria are early targets for Aβ aggregates and indicating that Aβ presence in mitochondria is an early stage of AD pathogenesis (Gillardon *et al.*, 2007; Ankarcrona, Mangialasche and Winblad, 2010). While the exact mechanism of Aβ neurotoxicity remains largely unknown, Zhang *et al.* (2012) identified a single proapoptotic protein, a member of the mitochondrial solute carrier family (SLC25), which interacts with APP and is associated with the characteristic neurodegeneration observed in AD. This study was conducted *in vitro* using yeast-two-hybrid assays identifying the SLC25A38 protein, which was assigned the name appoptosin, as the link between APP interaction and neuronal apoptosis (Zhang *et al.*, 2012). Until recently, there was no knowledge of the function of appoptosin. Guernsey *et al.* (2009) provided evidence that appoptosin is responsible for transporting glycine and 5-amino-levulinic acid (δ-ALA) across the mitochondria, vital for heme synthesis. A balance of free heme and protein-bound heme is maintained via homeostasis and maintains cell integrity. Alteration of this homeostatic balance can result in excess free heme which can lead to increased reactive oxygen species (ROS) and a destabilized mitochondrial cytoskeleton (Kumar and Bandyopadhyay, 2005), ultimately resulting in faulty heme metabolism. Appoptosin regulates intrinsic caspase-dependent apoptosis via heme biosynthesis, associating appoptosin with the neuronal death seen in AD and other neurodegenerative diseases (Zhang *et al.*, 2012). Using cultured rat hippocampal neurons it has been demonstrated that on exposure to low sub-cytotoxic levels of Aβ, severe impairment of mitochondrial transport (Rui *et al.*, 2006), increased mtDNA levels and increased numbers of malformed mitochondria can be observed (Diana *et al.*, 2008). Aβ binds to the Aβ-binding alcohol dehydrogenase protein in mitochondria, and by blocking this interaction both neuronal apoptosis induced by Aβ and generation of free radicals in neurons can be suppressed (Lezi and Swerdlow, 2012). These findings have also been confirmed in AD studies with human participants (Lustbader *et al.*, 2004; Caspersen *et al.*, 2005; Crouch *et al.*, 2005; Devi *et al.*, 2006).

The role of Aβ mitochondria has been investigated more since these studies, and research suggests that Aβ cannot be generated locally in mitochondria (Hansson-Peterson *et al.*, 2008), therefore it must be taken up by the organelle from elsewhere inside the cell. Using isolated rat mitochondria, it was demonstrated that Aβ is internalized by cells from an extracellular source and then imported into the mitochondria via the translocase of the outer membrane complex before accumulating in the mitochondrial cristae (Hansson-Peterson *et al.*, 2008).

Oxidative stress

Oxidative stress plays a key role not only in AD pathogenesis, but also in other neurodegenerative disorders such as Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease (Dias, Junn and Mouradian, 2013). The brain is particularly vulnerable to oxidative stress due its high oxygen demand, requiring 20% of the body's oxygen despite only making up approximately 2% of the body weight (Jain, Langham and Wehrli, 2010). In a 2-year study conducted from 2010 to 2012, a correlation was observed between oxidative stress and cognitive decline in AD in those aged 63–93 years, using glutathione as a biomarker for oxidative stress (Revel *et al.*, 2015). DNA bases are particularly vulnerable to damage caused by oxidative stress, which can trigger excitotoxic responses ultimately resulting in cell death (Feng and Wang, 2012).

As part of the electron transport chain (ETC), protons are pumped across the IMM from the mitochondrial matrix, resulting in a negative membrane potential across the IMM. This causes small numbers of electrons to slowly move out of the redox enzyme complexes (Ankarcrona, Mangialasche, and Winblad, 2010) in the IMM, which are capable of forming the superoxide radical (O_2^-), one of the main ROS, on interaction with oxygen molecules (Picone *et al.*, 2014). Mitochondria encompass a widespread and intense antioxidant defence mechanism in order to destroy any ROS, such as the superoxide radical, that may be formed during normal respiration. Any damage to mitochondria can result in interrupting the usual mechanisms by which ROS are destroyed, therefore increasing the number of ROS present in the organelle (Picone *et al.*, 2014). For example, it has been found that in post-mortem AD brains there is a deficit of cytochrome c oxidase (COX), the terminal enzyme in the mitochondrial respiratory chain responsible for reducing oxygen radicals, in the occipital, parietal, temporal and frontal lobes as well as in

the hippocampus (Mutisya, Bowling, and Beal, 1994). This dysfunction forms a cycle in which mitochondria contain more ROS due to damage, which in turn results in more damage due to an increase in the number of ROS present and so on. Excess free radicals can cause various biochemical changes observed in neurodegeneration, such as lipid peroxidation (Padurariu *et al.*, 2013). This results in cell damage and is responsible for some of the classic pathological changes observed in neurodegenerative disease.

Oxidative stress has also been found to alter Aβ levels and tau phosphorylation, the two key hallmarks of AD, via the modification of signalling pathways. Tau phosphorylation is increased via the activation of glycogen synthase kinase 3-β during high levels of oxidative stress (Lovell *et al.*, 2004), which also increases the expression of β-secretase (Tamagno *et al.*, 2005) and upregulates Aβ. Discovery of this link between oxidative stress in mitochondria and AD has sparked research into potential new drug targets, aimed at both oxidative stress and ROS.

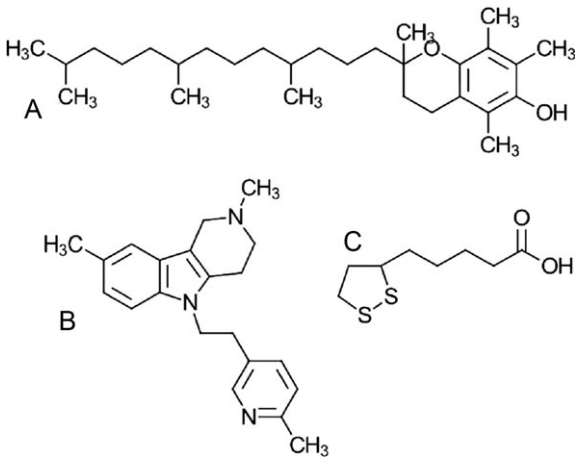

Figure 3. Chemical structures of α-tocopherol (A), Dimebon (B) and α-lipoic acid (C).

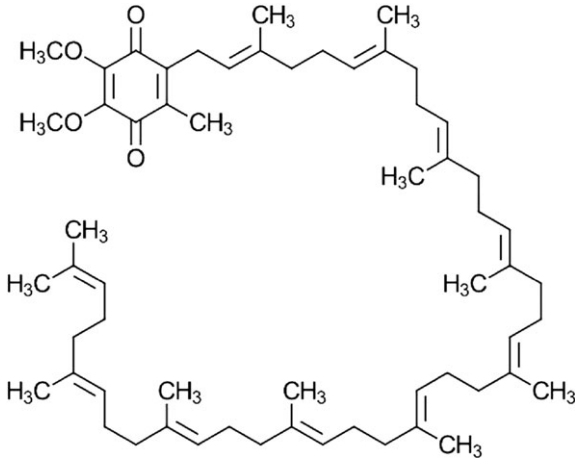

Figure 4. Chemical structure of coenzyme Q10.

Potential new treatment strategies

Antioxidants

Antioxidants bind with free radicals to diminish the latter's highly reactive and destructive properties and decrease the damage they cause. Antioxidants have been meticulously studied with regards to reducing mitochondrial toxicity caused by oxidative stress; however, even though there has been a great amount of research conducted in this area (Kumar and Singh, 2015) it has produced debatable results due to the low permeability of the blood brain barrier to many of the antioxidants used today (Picone *et al.*, 2014). New treatment strategies targeting this limitation have recently been tested, such as those using nanoparticles in order to deliver a more successful route for antioxidant drugs entering the central nervous system (Gomes, Martins and Sarmento, 2015).

Vitamin E is a generic term for a group of naturally occurring derivatives of tocopherol and tocotrienol, and is a crucial antioxidant in protecting cellular membranes such as those found in mitochondria (Ankarcrona, Mangialasche, and Winblad 2010). α-tocopherol (Toc) (Fig. 3A) is the most studied of these with regards to AD, and has been found to significantly slow the rate of cognitive decline in those with mild to moderate (Dysken *et al.*, 2014), albeit not being specifically targeted at mitochondria. However, in a study on aged male C57BL/6 J mice, the supplementation of one antioxidant alone had little or no effect in increasing cognitive function and it was shown that age-related cognitive dysfunction could be reversed by supplementing the mice with Toc and Coenzyme Q_{10} (CoQ) (Fig. 4), involved naturally in the respiratory chain in combination (Shetty *et al.*, 2014).

α-lipoic acid (LA) (Fig. 3B) is itself a powerful antioxidant which has the ability to recycle other antioxidants such as vitamins C and E. LA is a naturally occurring cofactor of mitochondrial enzymes α-ketoglutarate dehydrogenase and pyruvate dehydrogenase, and has been found to increase ACh production and scavenge the toxic products of lipid peroxidation (Maczurek *et al.*, 2008). For more than 30 years, LA has been used in Germany as a treatment for diabetic polyneuropathy (Ziegler *et al.*, 1999). However, it was not until an elderly patient already receiving LA treatment for diabetic polyneuropathy was diagnosed with early stage AD in 1997 that clinical trials began. When this patient was diagnosed with AD, AChEIs were prescribed as standard and her course of 600 mg per day of LA was continued. Over time her AD did not worsen as rapidly as expected, and her cognitive decline was found to be surprisingly slow (Hager *et al.*, 2001). In the subsequent clinical trial conducted by Hager *et al.* (2001), nine patients with probable AD were given 600 mg of LA daily, as well as either donepezil or

rivastigmine. Results demonstrated that cognitive decline was slowed in these patients after the LA was prescribed in comparison to AChEIs alone.

Many naturally occurring antioxidants have been tested as therapies for AD; however, most have failed to improve cognitive function. Promising new mitochondria-targeted antioxidants have been manufactured as a result of this. One of these specific antioxidants is mitoquinone mesylate, more commonly known as MitoQ (McManus, Murphy and Franklin, 2011). MitoQ accumulates *in vivo* and was specifically designed to protect the mitochondrial membrane from the severe damage that can be caused by lipid peroxidation and oxidative stress (Smith *et al.*, 2012). The main antioxidant component of MitoQ is ubiquinone, identical to the active antioxidant found in CoQ, which is selectively taken up by mitochondria due to the membrane potential produced, resulting in an almost thousand fold concentration of the drug inside the mitochondrial matrix (Ng *et al.*, 2014). The synthesis of MitoQ is carried out by covalently binding the ubiquinone component to a cation called decyltriphenylphosphonium through a long aliphatic carbon chain. The ubiquinone is then introduced to the lipid bilayer of the mitochondrial matrix where it is reduced rapidly to a product known as ubiquinol, the active antioxidant of MitoQ. This ubiquinol, once introduced, is recycled continuously via the ETC (Ng *et al.*, 2014) (Fig. 5). Since MitoQ is found in such high concentrations within the mitochondria, it is able to neutralize free radicals before they even reach their targets and thus drastically reduces the damage that these free radicals may cause (Picone *et al.*, 2014). Ng *et al.* (2014) treated transgenic *Caenorhabditis elegans* overexpressing human Aβ peptide, which leads to progressive paralysis in *C. elegans*, with MitoQ of varying concentrations in a blinded dose-response study. It was found that when administered both 1 and 5 μM MitoQ, *C. elegans* had significantly longer lifespans when compared to an untreated control. It was also found in a study by McManus, Murphy and Franklin (2011) that MitoQ had positive effects in a triple transgenic mouse model of AD, where MitoQ was able to prevent cognitive decline, oxidative stress, Aβ accumulation and synaptic loss in the brains of the mice.

Though this research has so far only been conducted in transgenic mouse models and *C elegans*, it demonstrates that mitochondria-targeted antioxidants such as MitoQ could have improved therapeutic potential when compared to natural antioxidants, and therefore could be successful treatment strategies with regards to AD and similar neurodegenerative diseases in the future.

Dimebon (Latrepirdine)

Latrepirdine, sold as dimebon (Fig. 3C), is a drug that has been used clinically in Russia as a non-selective antihistamine for skin allergies and allergic rhinitis since 1983 (Ankarcrona, Mangialasche and Winblad, 2010), but was withdrawn to be used for more selective treatments. The exact mechanism by which dimebon works is not yet known (Perez *et al.*, 2012); however, in several *in vitro* studies, dimebon has shown to be neuroprotective against $A\beta_{25\text{-}35}$ β-amyloid fragments in cerebellar granule cell cultures, designed to mimic the neurodegeneration found in the likes of AD (Bachurin *et al.*, 2001; Lermontova *et al.*, 2001). Dimebon has also been found to be a weak AChEI, with a half maximal inhibitory concentration (IC_{50}) of 8–42 μM (Bachurin *et al.*, 2001; Schaffhauser *et al.*, 2009). It has also proved to be a weak NMDA receptor antagonist (IC_{50} = 10 μM) (Grigorev, Dranyi and Bachurin, 2003; Schaffhauser *et al.*, 2009) and a weak inhibitor of voltage-gated Ca^{2+} channels (IC_{50} = 50 μM) (Lermontova *et al.*, 2001; Schaffhauser *et al.*, 2009). At an optimum concentration of 10 μM, dimebon has an inhibitory effect of more than 50% on a total of 18 receptors, including several serotonin receptors (Schaffhauser *et al.*, 2009).

The main component of the tau inclusions found in frontotemporal lobar degeneration, a form of dementia similar to AD characterized by severe muscle wasting, is a protein known as transactivation responsive DNA binding protein of 43 kDa (TDP-43) (Yamashita *et al.*, 2009). This protein inclusion has also been found in a subpopulation of patients with other neurodegenerative diseases such as AD and dementia with Lewy bodies (Arai *et al.*, 2009) as well as Huntington's disease (Schwab *et al.*, 2008). In an *in vitro* study conducted on neuroblastoma SH-SY5Y cells expressing mutated TDP-43, it was found that 5 μM concentrations of dimebon over a 3-day incubation period reduced the number of TDP-43 aggregates by 45% when compared to controls (Yamashita *et al.*, 2009), suggesting that dimebon possesses anti-oligomerization properties. Numerous trials have been conducted using dimebon as a possible therapy for AD, as was carried out by Doody *et al.* (2008). In this randomized, double-blind, placebo-controlled phase II trial, 183 Russian patients with mild to moderate AD were randomly assigned either 60 mg of dimebon per day or a matched placebo. Results showed a significant improvement in cognitive function and a significantly increased score on CFTs in the dimebon group compared to the placebo (Doody *et al.*, 2008).

These findings showed promise for dimebon as a potential AD therapy, sparking further study. Thus, an international double-blind phase III CONNECTION trial was conducted in 2010 using 598 participants from North America, South America and Europe. When dimebon was compared to a placebo group over a 6-month period, patients with mild to moderate AD did not show a statistically higher performance on any CFTs, and therefore dimebon did not meet CONNECTIONs co-primary (cognition and global function) or secondary efficacy end points (Pfizer Inc. and Medivation Inc., 2010). The plausibility of dimebon has been questioned by some as a treatment for AD, and it is not yet Food and Drug Administration approved as an AD treatment. Further phase III trials are ongoing in an attempt to replicate the findings of Doody *et al.*'s (2008) study. One randomized, double-blind, placebo-controlled phase III trial known as CONCERT enrolled approximately 1050 participants with mild to moderate AD from numerous sites across Western Europe, USA, Australia and New Zealand

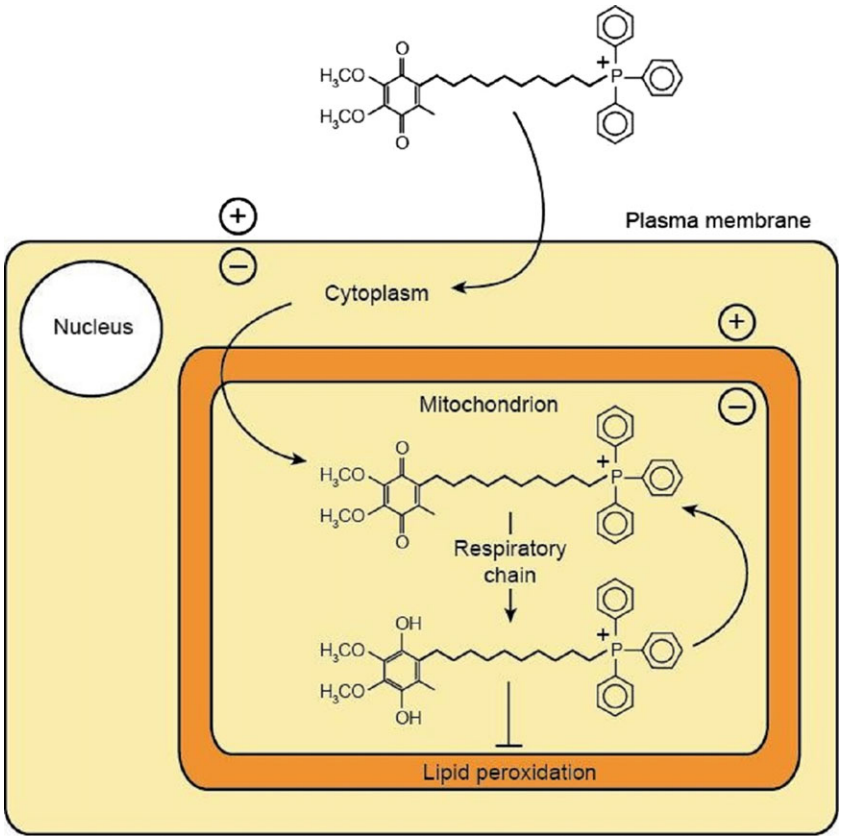

Figure 5. Mechanism of MitoQ entering the cell and the mitochondria, with the recycling of MitoQ via the ETC displayed. (Reproduced from MitoQ.com, open access under the Creative Commons Attribution License CC-BY-SA 3.0.)

to participate in a 12-month study, designed to evaluate the efficacy of dimebon when added to ongoing AD treatment with donepezil (Pfizer Inc. and Medivation Inc., 2015).

The results of this trial were unfortunately negative yet again, which proves detrimental for dimebon as an AD therapy, and further highlights the difficulties scientists face in finding potential treatments for diseases like AD.

Conclusion

Mitochondrial dysfunction is an early feature of AD pathology. Any damage to mitochondria by either Aβ or ROS can result in interrupting the usual mechanisms by which ROS are destroyed, therefore further increasing the number of ROS present in the organelle. It has been found that in post-mortem AD brains there is a deficit of COX, the terminal enzyme in the mitochondrial respiratory chain responsible for reducing oxygen radicals, supporting the theory that ROS are involved in the mitochondrial damage found in AD. Many antioxidants have been investigated as therapies for AD, and further studies have been carried out to find mitochondria specific antioxidants to enable a greater concentration of antioxidants to accumulate in the mitochondria, allowing a more specific method for combatting mitochondrial oxidative stress. It has been suggested that as early as 2025, prevention or effective treatment of AD may be realized (Cummings *et al.*, 2016). Steps along this road include finding therapies that inhibit primary progenitors, reduce secondary symptoms, slow AD progression and ultimately repair damaged neurons (Feng and Wang, 2012).

Further study of both generic and mitochondria specific antioxidants should be carried out to find more potential treatment strategies for Aβ induced neurotoxicity in AD and other neurodegenerative diseases.

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Author biography

Zoe L. Hawking recently graduated the University of Sunderland's BSc Biomedical Sciences degree in July 2016 with a first class honours. This review is largely unmodified from her level 5 biosciences literature review. Zoe has

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