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Promotion of Cellular NAD+ Anabolism: Therapeutic Potential for Oxidative Stress in Ageing and Alzheimer's Disease

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Oxidative imbalance is a prominent feature in Alzheimer's disease and ageing. Increased levels of reactive oxygen species (ROS) can result in disordered cellular metabolism due to lipid peroxidation, protein-cross linking, DNA damage and the depletion of nicotinamide adenine dinucleotide (NAD^+). NAD^+ is a ubiquitous pyridine nucleotide that plays an essential role in important biological reactions, from ATP production and secondary messenger signalling, to transcriptional regulation and DNA repair. Chronic oxidative stress may be associated with NAD^+ depletion and a subsequent decrease in metabolic regulation and cell viability. Hence, therapies targeted toward maintaining intracellular NAD^+ pools may prove efficacious in the protection of age-dependent cellular damage, in general, and neurodegeneration in chronic central nervous system inflammatory diseases such as Alzheimer's disease, in particular.

Keywords: NAD^+, Alzheimer's disease; Inflammation; Neurodegeneration; Oxidative Stress; DNA repair

INTRODUCTION

Alzheimer's disease (AD) represents the most common neurological disorder in old age (Maccioni *et al.*, 2001). Over 24 million people have dementia worldwide, of which more than 60% is due to AD (Ferri *et al.*, 2006). The prevalence of this progressive, neurodegenerative disorder increases exponentially with age, reaching one in five by the age of eighty (Evans *et al.*, 1989; Skoog *et al.*, 1993). The major pathological hallmarks of AD include the formation of extracellular Amyloid-beta (Aβ) deposits called senile plaques; and twisted intracellular bundles of fibre known as neurofibrillary tangles (NFT) (Mattson, 2004; Parker, 2004). These lesions are initially associated with progressive loss of cholinergic neurons in the temporal and frontal lobes of the brain (Mattson, 2004; Parker, 2004).

The important question concerning AD is why only selected neuronal cells are targeted for destruction, particularly in the early stages of the disease. Researchers over the last two decades have identified several genes, toxins, proteins and metabolic abnormalities associated with the development of AD (Suh and Checler, 2002). However the key to unlocking an effective treatment or prevention strategy has so far remained elusive. There is a growing confluence of opinion that oxidative stress may serve as a point of convergence for the potentially mixed aetiology of AD and is responsible for most of the neuronal pathology seen in the AD brain (Floyd and Hensley, 2002; Citron, 2004;

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Mattson, 2004). It is possible then that currently unrecognised downstream effects of ROS activity may act as a primary trigger for neuronal cell death.

Low levels of oxidative stress occur continuously throughout the body as part of normal cell metabolism (Ogino and Wang, 2007), and their effects are minimised by constitutive antioxidant and repair processes. However accelerated production of ROS such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet -}$), and hydroxyl radical ($HO^{\bullet}$) will cause pathological damage to the cell and its DNA. Efficient repair mechanisms are therefore essential to ensure the avoidance of cell death. Importantly, the essential pyridine nucleotide NAD^+ has been identified as a key element in both DNA repair and gene signalling pathways (Erdelyi *et al.*, 2005; Malanga and Altaus, 2005; Lin and Yang, 2007).

Historically NAD^+ was identified as the parent compound of the pyridine family of coenzymes (NADH, $NADP^+$, NADPH) that act as essential cofactors for several enzyme-catalysed oxidative reactions including alcohol, lactate and amino acid metabolism. NAD^+ also serves as an electron transporter to power oxidative phosphorylation and energy (ATP) production, and plays a key role in glutathione metabolism and the NADPH-dependent thioredoxin system, which are involved in the maintenance of the endogenous antioxidant defense system and detoxification reactions (reviewed in Parihar and Brewer, 2007). However, it has recently been shown that NAD^+ is also actively involved in a number of other important cell functions including DNA repair, gene silencing and cellular energy-sensing (Rafaeloff-Phail *et al.*, 2004).

In genomic DNA repair NAD^+ is the sole substrate for the DNA nick sensor poly (ADP-ribose) polymerase (PARP). The PARP family of enzymes, in particular PARP-1, are DNA binding enzymes activated by ROS initiated breaks to the DNA and are critical to the base excision repair (BER) process (Bouchard *et al.*, 2003). While many proteins are involved in repairing DNA damage, the majority of lesions are repaired by BER (Bouchard *et al.*, 2003). PARP-1 and its essential substrate, NAD^+, are therefore critical to the DNA repair process following oxidative damage and hence continuing cell survival. Rapid NAD^+ depletion through PARP activation following ROS insult can precipitate cellular energy crisis through a reduction in ATP synthesis resulting in cell death (reviewed in Pacher and Szabó, 2007) in a number of cell types (Pillai *et al.*, 2005) including the brain (Alano *et al.*, 2004).

In addition to its role in, PARP activity, NAD^+ also serves as a substrate for a new class of enzymes known as the sirtuin family, or silent information regulators of gene function (Sauve *et al.*, 2006). Gene silencing by this family of enzymes has been correlated directly with longer lifespan (Yang and Sauve, 2005). As sirtuin function involves cleavage of the glycosidic bond between nicotinamide and the adenosine 5'-diphosphoribose (ADPR) moiety, continuous biosynthesis of NAD^+ is vital to the maintenance and ongoing function of all cells (Berger *et al.*, 2004).

While the precise cause of neuronal cell death in AD is not known its mediation through inflammatory changes involving ROS and accelerated DNA damage appear central to cellular AD pathology. As the number of individuals with dementia is expected to triple by 2050, and that current therapeutic strategies are ineffective at halting disease progression, new effective intervention strategies are urgently needed (Mattson, 2004).

In this review we will consider the role of oxidative stress in the development of neuronal pathology in AD and discuss its link with DNA damage and changes in NAD^+ levels and their relationship to continuing cell viability. NAD^+ plays a central role in maintaining cellular energy (ATP), DNA repair and numerous other essential cell functions. The intracellular pool of NAD^+ can be rapidly depleted during oxidative stress as occurs in neurodegenerative diseases such as AD. Promotion of NAD^+ synthesis may therefore prove a promising therapeutic strategy for the treatment of some neurodegenerative disorders.

OXIDATIVE IMBALANCE IN AD AND AGEING

Several hypotheses have been devised to account for the aetiology of AD, all of which share the commonality of oxidative stress. The significance of oxidative stress in the natural history of the disorder is consistent with ageing being a major risk factor for the disease (Mates, 2000; Floyd and Hensley, 2002).

ROS such as HO·, $O_2^{\cdot -}$, and H_2O_2 are formed as by-products of respiration and oxidative metabolism (Mates, 2000). Under pathological conditions, detoxifying enzymes such as the glutathione peroxidase (GTP) and superoxide dismutase (SOD), together with antioxidant mechanisms such as vitamins A, C and E form the essential components of the defense system against free radical injury (Halliwell, 1992). An imbalance between the formation of free radicals due to increased ingestion or synthesis of radical-generating toxins, and the protective mechanisms appear to be a major factor not only for 'normal' ageing, but also for the pathological processes involved in the development of AD (Retz *et al.*, 1998).

The central nervous system (CNS) is extremely vulnerable to free radical mediated destruction due to significant oxidative metabolic activity, lower levels of antioxidants and protective enzymes, and an abundance of polyunsaturated fatty membranes (Mates, 2000). Since most neurons do not proliferate, the CNS neural network can be readily disrupted (Martin *et al.*, 1999). Importantly, the aged have an increased susceptibility to oxidative stress due to generally negative changes in dietary patterns, absorption or utilisation of nutrients, or underlying infections, which can disrupt the normal antioxidant defense system (Martin *et al.*, 1999).

A major factor associated with age-related diseases is the increase in oxidative DNA damage (Miquel, 1992). DNA damage may affect the expression of a variety of genes involved in the regulation of cell proliferation or inhibit expression of other genes associated with DNA repair (Gravey *et al.*, 1999). Many cell types do not show time-related degeneration due to rapid turnover. However, some mammalian cells, particularly neurons change considerably with age (Miquel, 1992). Elevated levels of HO·-mediated DNA and RNA damage are consistently observed in the AD brain (Nunomura *et al.*, 1999). As HO· only diffuse across nanometer distances these radical species must be generated in close proximity to the site of intracellular damage (Zhu *et al.*, 2005).

SOURCES OF ROS IN AD

Generation of oxygen free radicals have been closely associated with extracellular Aβ, redox-active transition metals, and mitochondrial abnormalities (Zhu *et al.*, 2005). In addition, it has recently been shown that over-activation of the *N*-methyl D-Aspartate (NMDA) receptor by quinolinic acid (QUIN) in the AD brain significantly elevates free radical production, likely adding to A 's free radical induced damage (Guillemin and Brew, 2002).

β-Amyloid, Redox active metals (Fe^{2+} and Cu^{+}) and ROS

Aβ has become central to the leading hypothesis of AD pathogenesis since its isolation from AD plaques more than two decades ago (Masters *et al.*, 1985), playing a key role in the development of neurofibrillary tangles (Oddo *et al.*, 2006) and oxidative stress. Aβ is a 4kDa hydrophobic protein that is derived from the endocytosis of the larger amyloid precursor protein (APP) (Glenner and Wong, 1984; Masters *et al.*, 1985). The two major carboxyl terminal modifications of Aβ include $Aβ_{1-40}$, and $Aβ_{1-42}$ (Masters *et al.*, 1985). The former is the major species secreted by cell cultures and in the CSF while the latter variant is the principle component of amyloid plaques in the AD brain and is more prone to aggregation (Younkin, 1995).

Aggregation of APP-derived Aβ into protease fibrils is thought to be a significant step in the development of AD (Tjernberg *et al.*, 1999). Butterfield (1997) proposed that insertion of these soluble aggregates into neuronal and glial bilayer membrane could generate H_2O_2 as a major ROS (Varadarajan, 2000). The reduction of metal ions such as iron (II) (Fe^{2+}) in the presence of H_2O_2 by the Fenton reaction ($Fe^{2+} + H_2O_2$ --> Fe^{3+} + HO· + HO-) can subsequently produce the highly reactive HO· resulting in significant damage to the cell (Huang *et al.*, 1999).

In the human body the accumulation of Fe^{2+} in the systemic circulation is prevented by the presence of binding proteins, such as ferritin and transferrin (Miranda *et al.*, 2000). However, the brain is limited in its ability to regulate the levels of Fe^{2+} through these mechanisms, allowing potential metal-induced cellular damage to accumulate over time (Miranda *et al.*, 2000).

Metal ion dyshomeostasis has been observed in the AD brain and likely plays a crucial role in the death of neuronal cells (Liu *et al.*, 2006). Elevated

Fe^{2+}concentrations have been observed in the cytoplasm of vulnerable neurons in the amygdala and hippocampus of AD patients (Smith *et al.*, 1997). The copper concentration in the CSF is doubled in AD patients (Behl *et al.*, 1994). NFTs and senile plaques, (hallmark pathologies of AD), contain both Fe^{2+} and Cu^{+} (Doraiswamy and Finefrock, 2004). Together, these metals can promote in-vitro Aβ aggregation, and amyloid formation under slightly acidic conditions, and act as a cofactor to Aβ, further generating ROS (Doraiswamy and Finefrock, 2004).

Several destructive processes induced by ROS have been observed following exposure of cells to Aβ through (1) increased intracellular calcium $[Ca^{2+}]_i$ via N- and L- type voltage dependent calcium channels and further potentiation of oxidative stress through the increased production of phospholipase and arachidonic acid and subsequent production of oxygen radicals via fatty acid metabolism (Kontos, 1989; Kim *et al.*, 2000); (2) inhibition of glutamate synthetase (GS) activity in astrocytes resulting in excess accumulation of the excitatory neurotransmitter, glutamate, and the aggravation of excitotoxicity (Harris *et al.*, 1995); and (3) reduction in glucose uptake in neurons and vascular endothelial cells by reducing the number of the glucose transporter protein (GLUT1) (Blanc *et al.*, 1997; Horwood and Davies, 1994) with the resultant hypoglycaemia exposes cells to subsequent excitotoxic damage (Martin *et al.*, 1999).

Increased intranuclear levels of Fe^{2+} has been implicated as a potential source of ROS-mediated DNA damage and NAD^{+} depletion in AD (Wolozin and Golts, 2002; Jayasena *et al.*, 2007). Metal-chelating agents may be useful in reducing the generation of H_2O_2 by Aβ, and prevent Aβ aggregation, DNA damage, and subsequent depletion of the essential substrate and cofactor, NAD^{+} (Cherny *et al.*, 2001; Regland *et al.*, 2001; Jayasena *et al.*, 2007).

Mitochondrial Abnormalities

Mitochondrial abnormalities have also been implicated in the pathophysiology of AD. Indeed, the activities of several mitochondrial enzymes of oxidative metabolism are reduced in the AD brain, including α-ketoglutarate dehydrogenase (KGDHC), and pyruvate dehydrogenase (PDHC) (Gibson *et al.*, 2000). Since these enzymes are actively involved in the reduction of molecular oxygen, reduced activity promotes the generation of ROS, particularly superoxide (Zhu *et al.*, 2005).

Studies have shown Cu, Zn-superoxide dismutase activity (SOD1), the main enzyme that facilitates the removal of superoxide anions, $O_2^{\bullet -}$, by converting them to H_2O_2 is elevated in the AD patients (Serra *et al.*, 2001; Ozcankaya and Delibas, 2002). Increased $O_2^{\bullet -}$ levels coupled with elevated SOD1 activity can therefore generate high amounts of H_2O_2, which can diffuse more readily across the outer membrane of the mitochondria and accumulate in the cytoplasm and the nucleus. As mentioned previously, H_2O_2 can react with transition metals leading to markedly increased $HO^{\bullet}$ production resulting in chronic and significant DNA damage.

Furthermore, point mutations in several mitochondrial genes have been observed in the AD brain (reviewed in Parihar and Brewer, 2007). One study reported reduced NADH dehydrogenase activity, a component of the respiratory chain complex I, in the homogenates of Aβ plaques. As mitochondrial NADH dehydrogenase is actively involved in re-oxidising NAD^{+}, this study suggests that the ability to regulate the NAD^{+}:NADH ratio is reduced in AD patients (Lin and Guarente, 2003). This again supports the notion that reduced function of critical NAD^{+} dependent biochemistry may contribute to AD pathology.

Quinolinic Acid and the Kynurenine Pathway

There is growing evidence implicating the kynurenine pathway (KP) as an additional factor in neurodegenerative processes in AD (Guillemin *et al.*, 2002; Finkbeiner and Cuero, 2006). The KP is the principle route of L-tryptophan catabolism, resulting in the production of NAD^{+} and several neuroactive intermediates (Stone, 2001). Of particular interest is the selective NMDA receptor agonist, QUIN (Stone, 2001).

Senile plaques in the AD brain are associated with evidence of chronic local inflammation mediated by both microglia and macrophages (Guillemin and Brew, 2002). Guillemin *et al.* (2003; 2007) demonstrated that $A\beta_{1\text{-}42}$ induces the production of QUIN by macrophages and microglia in neurotoxic concentrations. Moreover, immunohistochemical studies have shown an up-regulation of QUIN produc-

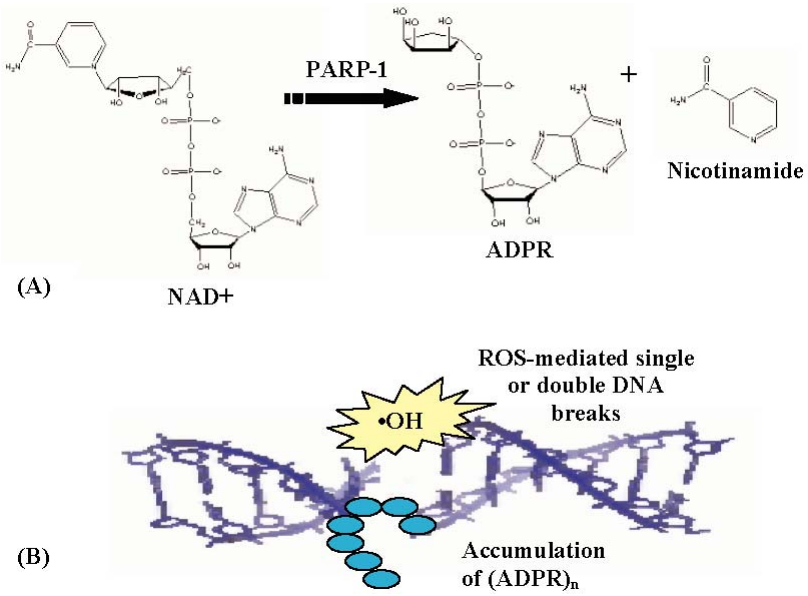

FIGURE 1 (**A**) Stoichiochemistry for Poly-ADP-ribosylation. (**B**) Accumulation of Poly ADP-ribose following ROS-mediated double strand DNA breaks.

tion in association with senile plaques in the AD brain (Guillemin and Brew, 2002; Guillemin *et al.*, 2005). Acute exposure to QUIN at pathophysiological concentrations can lead to neuronal cell death while chronic exposure causes metabolic and functional disruption to neurons, culminating in cell death via an apoptotic mechanism (Heyes, 1993; Kerr *et al.*, 1995; 1998; Guillemin and Brew, 2002).

A major aspect of QUIN mediated neurotoxicity is the enhanced formation of ROS and consequent oxidation of lipids in the AD brain (Rios and Santamaria, 1991; Santamaria *et al.*, 1997; Behan *et al.*, 1999; Guillemin and Brew, 2002).

Stimulation of NMDA receptors is well known to activate Na^+-K^+ ATPase and Ca^{2+}-ATPase enzymes, leading to greater ATP demand. The increase in energy requirement, together with increased Ca^{2+} influx generating large amounts of ROS including nitric oxide (NO·). This process can lead to significantly higher DNA damage, PARP activation and NAD^+ depletion, culminating in cell death due to energy deprivation (reviewed in Parihar and Brewer, 2007).

EFFECT OF CHRONIC OXIDATIVE STRESS IN DNA REPAIR

Oxidative stress-mediated damage to DNA, lipids, and proteins has been shown to accumulate during AD and increase with age. A key mechanism for repair of DNA strand breaks involves the DNA base excision repair enzyme, poly(ADP-ribose) polymerase (PARP) (Meyer *et al.*, 2006). As indicated previously the PARP family represents a group of intracellular enzymes dependent on available nuclear NAD^+ as essential substrate. While actions of PARP-1 have long been recognized, several other PARP enzymes (PARP 2 to PARP 7) have recently been associated with the maintenance of genomic integrity (Meyer *et al.*, 2006).

Activation of PARP catalyses the cleavage of NAD^+ into adenosine 5'-diphosphoribose (ADPR) and nicotinamide, and the covalent attachment of polymers of ADP-ribose to histones and other nuclear proteins, including PARP itself (de Murcia *et al.*, 1997). PARP-1, which accounts for the majority of the ADPR synthesised, is found at the highest concentrations in the nucleus (Meyer *et al.*, 2006) (FIG. 1).

PARP activation leads to DNA repair and recovery of normal cellular function (Meyer *et al.*, 2006). Experimental studies have shown that PARP is activated in response to free radical-mediated injury to DNA after brain ischemia and reperfusion (Zhang *et al.*, 1994). Hyperactivation of PARP following DNA strand breaks can rapidly consume intracellu-

lar NAD^+ pools, resulting in loss of the ability to synthesise ATP, and cessation of all energy dependent functions, with consequent cell death (di Lisa and Ziegler, 2001; Wang *et al.*, 2003; Meyer *et al.*, 2006). As discussed in more detail later in this article, excessive activation of PARP in response to oxidative damage can also decrease SIRT1 deacetylase function through NAD^+ depletion, resulting in the accumulation of acetylated p53, leading to cell death via the induction of p53 apoptotic mediators (Furukawa *et al.*, 2007). Maldonado *et al.* (2007) also showed that PARP activation plays an active role in neuronal death induced by QUIN in rats.

PARP mediated NAD^+ depletion has been implicated in the pathogenesis of AD (Love *et al.*, 1999), with one study showing that poly(ADP-ribose) polymers accumulate at higher concentrations in the temporal and frontal cortex in the brains of AD patients compared to control brains. This indicates that PARP is over-expressed in the AD brain, and implies an excessive NAD^+ turnover in susceptible neuronal cells (Love *et al.*, 1999).

Therefore, maintaining an adequate intracellular NAD^+ supply appears essential for the maintenance of optimal DNA repair and cellular function during conditions of chronic oxidative stress.

IMPORTANCE OF MAINTAINING INTRACELLULAR NAD^+ LEVELS

Numerous studies have shown that NAD^+ turnover is increased during oxidative stress (Grant and Kapoor, 1998; Furukawa *et al.*, 2007; Ying, 2007). As AD pathology involves a chronic increase in ROS activity and subsequent PARP activation, NAD^+ turnover is likely to be significantly increased. Does the efficiency by which neurons synthesise new NAD^+ play a role in the selective neuronal cell death during chronic CNS inflammatory disorders such as AD?

ATP Production

There is a well established role for NAD^+ as an essential electron acceptor in several steps in the glycolytic reaction sequence culminating in the generation of two molecules of ATP through substrate phosphorylation of phosphoglycerate kinase (PGK) and pyruvate kinase (Berg *et al.*, 2007). It has been demonstrated that excessive oxidative damage to DNA can have a deleterious effect on carbohydrate metabolism. As previously stated, excessive activation of PARP leads to depletion of intracellular NAD^+ pools, resulting in decreased oxidative phosphorylation and reduced ATP synthesis (Berger *et al.*, 1986).

The ultimate depletion of NAD^+ and ATP causes fatal alterations in carbohydrate metabolism. Vulnerable cells lose their ability to carry out energy dependent functions, including the maintenance of cell wall integrity and DNA/RNA synthesis and consequently die.

Secondary Messenger Signalling

In addition to the traditional release of intracellular Ca^{2+} by inositol triphosphate (IP_3), a further NAD-dependent Ca^{2+}-releasing process has been identified (Lee, 2001). Cytosolic NAD^+ and its phosphorylated form, nicotinamide adenine dinucleotide phosphate ($NADP^+$) can be converted to the Ca^{2+}-utilising protein, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), a reaction catalysed by NAD^+ glycohydrolase (NADase). NAADP stimulates intracellular Ca^{2+} release from lysosome-related organelles independent of the cytosolic $[Ca^{2+}]_i$ concentration. The Ca^{2+} is then mobilised by cADPR from the endoplasmic reticulum (ER) by a Ca^{2+}-induced Ca^{2+} release mechanism similar to IP_3-mediated Ca^{2+} release to generate ordered Ca^{2+} waves (Berger *et al.*, 2004; Lee, 2001). Therefore, NAD^+ depletion following conditions of oxidative stress may strongly influence $[Ca^{2+}]_i$ influx, disrupting essential Ca^{2+}-dependent cellular processes including the maintenance of normal membrane permeability and neuromuscular excitability (Parker, 2004) (FIG. 2).

PARP Activity and p53

As indicated earlier, NAD^+ is used as a substrate by PARP in DNA repair. Excessive activation of PARP following oxidative damage depletes the intracellular NAD^+ pool resulting in cell death by an apoptotic mechanism. By preventing NAD^+ depletion, ATP stores are preserved along with energy dependent cellular functions. PARP inhibitors have been shown to reduce neuronal cell death in animal models of stroke, traumatic brain injury, and Parkinson's disease (Jagtap and Szabo, 2005).

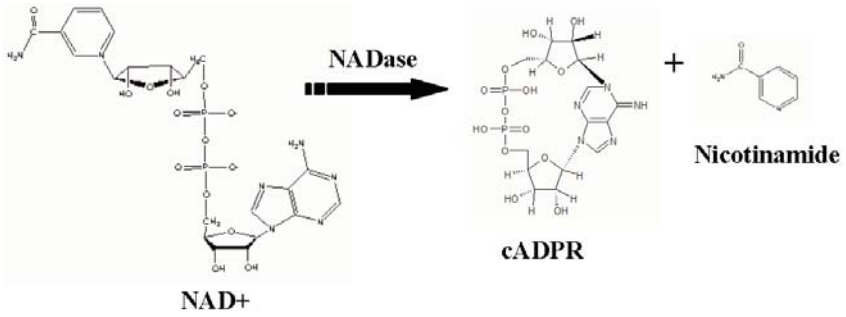

FIGURE 2 NAD glycohydrolase (NADase) regulates intracellular calcium release through synthesis of cADPR.

However, disabling PARP significantly reduces DNA repair capacity in vulnerable cells, with some cell death still occurring due to genotoxic recognition of un-repaired cell damage (Beneke *et al.*, 2004).

More recent work has highlighted the important role of PARP-1 in centrosome function (Miwa *et al.*, 2006). The centrosome acts as a microtubule organising centre and regulates cellular morphology and intracellular transport of cellular proteins (Doxsey, 2001). Importantly, the centrosome plays a vital role in maintaining chromosomal stability (Doxsey, 2001). Abnormal numbers of centrosomes, and abnormal spindles were observed in mitoses of mouse embryonic fibroblasts following administration of the PARP inhibitor 3-aminobenzamide to cell cultures (Miwa *et al.*, 2006).

PARP-1 interaction with important cell cycle regulators, such as p53 highlights the dynamic role of PARP enzymes in DNA damage surveillance and regulation of cell division (Alvarez-Gonzalez *et al.*, 2006). The main tumour suppressor protein p53 is induced by several cellular stressors including oxidative damage (Malanga *et al.*, 1998). It regulates the expression of several gene products that either lead to cell cycle arrest in G1 and prevent DNA replication immediately before the repair of damage, or cause cell death via an apoptotic mechanism (Malanga *et al.*, 1998).

Increased levels of PARP activity and p53 expression have been observed in degenerating neurons in AD patients and in cultured neurons treated with Aβ (de la Monte *et al.*, 1998; Culmsee *et al.*, 2001). PARP appears to play a positive role in the up regulation of p53. For example, cell lines derived from Chinese hamster V79 cells that were deficient in poly(ADP-ribosyl)ation failed to activate p53 in response to treatment with etoposide (Whitacre *et al.*, 1995). *In vitro* studies have shown that PARP can activate DNA dependent protein kinase and therefore regulate p53 activity through phosphorylation (Ruscetti *et al.*, 1998).

Altogether, PARP appears to play a beneficial role in the regulation of several cellular functions including DNA repair, centrosome function, and p53 expression. Therefore, pharmacological interventions that promote intracellular NAD^+ synthesis, (rather than inhibit PARP activity), will result in optimal PARP activity following DNA damage, thereby enhancing cellular resilience during conditions of oxidative stress and genotoxicity.

Sirtuin Activity

Sirtuins are a family of protein modifying enzymes that regulate key pathways in diverse organisms, from *Archaean* to humans (Sauve *et al.*, 2006). The founding member of the sirtuin family, Sir2, has been shown to deacetylate lysine residues in an NAD^+ dependent reaction that releases nicotinamide, acetyl ADP ribose (AADPR), and the deacetylated substrate (Sauve *et al.*, 2006), (FIG. 3).

Sirtuins have been shown to play a vital role in promoting cell survival in response to oxidative stress through caloric restriction regimens (Sauve *et al.*, 2006). In *Caenorhabditis elegans*, caloric restriction stimulated the activity of Sir2 (Yang and Sauve, 2005). It is thought that caloric restriction operates by increasing the free NAD^+ pool by elevating the NAD^+:NADH ratio and thus activating Sir2 (Sauve *et al.*, 2006).

Increased NAD^+ biosynthesis may therefore be associated with increased life span and transcriptional silencing at both telomeres and ribosomal DNA (Anderson *et al.*, 2002). Silencing of these

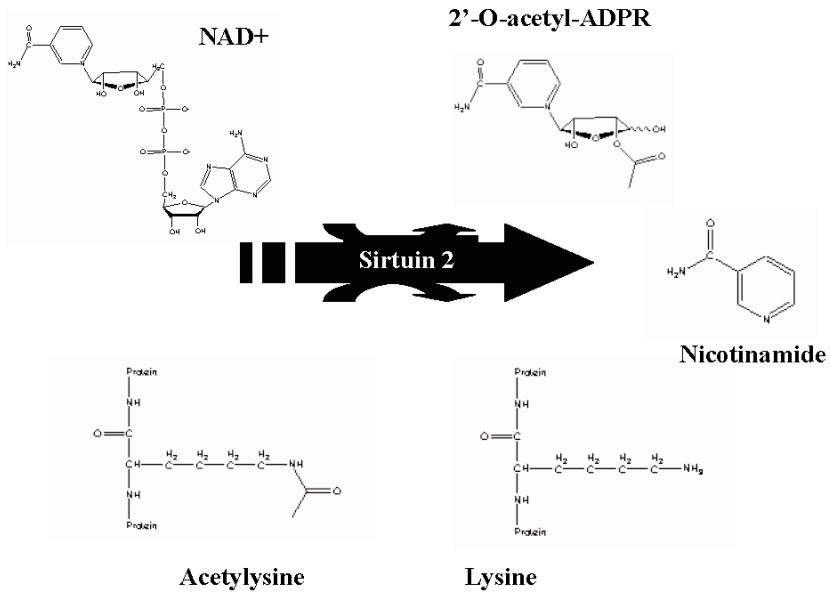

FIGURE 3 Stoichiochemistry for Sirt2 deacetylation.

loci is considered a measure of Sir2 activity, which requires NAD^+ (Anderson *et al.*, 2002). Therefore, manipulation of the intracellular NAD^+ pool at the genomic level may potentially enhance longevity.

SIRT1 is the mammalian homolog of Sir2. Consistent with observations in lower order organisms a number of beneficial effects have been observed in mice that over-express SIRT1. Bordone and colleagues (2007) found that these transgenic mice displayed phenotypes similar to mice on a calorie-restricted diet: including reduced body weight, greater metabolic activity, reduced blood cholesterol, adipokines, insulin and fasted glucose; and were more glucose tolerant.

Activation of sirtuins can extend the lifespan of cultured human cell lines by stimulating SIRT1-dependent deacetylation of p53 (Howitz *et al.*, 2003). Increased SIRT1 activity in human cells can delay apoptosis and provide vulnerable cells with additional time to repair them after repeated exposure to oxidative stress, and therefore limit unnecessary cell death (Howitz *et al.*, 2003).

The life-enhancing properties of sirtuins suggest that they may play a protective role against neurodegeneration, and may be considered a target for the treatment of age-related diseases such as AD (Porcu and Chiarugi, 2005). Maintaining adequate levels of the essential SIRT1 substrate NAD^+ will be integral to the success of this process.

Role of NAD^+ in AD and Aging

Considering the importance of NAD^+ in energy metabolism, DNA repair and transcriptional regulation, maintaining intracellular NAD^+ reserves emerges as a major therapeutic target for the treatment of several age-related degenerative diseases, including AD (Belenky *et al.*, 2007). In particular, increased nuclear NAD^+ biosynthesis and consequent activation of SIRT1 has been shown to protect mouse neurons from mechanical and chemical injury (Bedalov and Simon, 2004).

Over-expression of several enzymes of the NAD^+ salvage pathway, including nicotinate phosphoribosyltransferase (PNC1) and nicotinamide mononucleotide adenylyltransferase1 (NMNAT1) have been shown to extend lifespan in rat models by activating SIRT1 and promoting p53 deacetylation (Arraki *et al.*, 2004; Berger *et al.*, 2004; Bedalov and Simon, 2004; Porcu and Chiarugi, 2005; van der Veer *et al.*, 2007). As 1:1 stoichiometry exists between the intracellular NAD^+ content and sirtuin-mediated deacetylation (Grozinger and Schreiber, 2002), promotion of NAD^+ anabolism appears as an important therapeutic target for promoting sirtuin function in neuronal cells during periods of repeated oxidative stress observed in AD.

Increased NMNAT1 activity has also been shown to protect against axonal degeneration in Wallerian

degeneration slow (Wld^S) mice (Arraki *et al.*, 2004). Exogenous administration of NAD^+ prior to axotomy also delayed axonal degeneration, but to a lesser extent in NMNAT1 expressed mice, further indicating the importance of maintaining intracellular NAD^+ pools as a preventive measure against axonal degradation (Arraki *et al.*, 2004; Bedalov and Simon, 2004). In the absence of exogenous NAD^+, PARP inhibition increased the survival of dorsal root ganglion cultures following mechanical injury. No protective effect on Wld^S mice was observed following PARP inhibition in the presence of exogenous NAD^+ (Arraki *et al.*, 2004; Bedalov and Simon, 2004). This suggests that adequate intracellular NAD^+ levels are essential for neuronal survival.

Axonopathy is a critical feature of several neurodegenerative diseases and often precede the death of neuronal bodies in AD (Raff *et al.*, 2002). As axonal deficits are central to the patient's neurological disability, therapies that prevent axonal degradation are of great therapeutic importance for treating AD.

Changes in intracellular NAD^+ levels may also affect gene expression (Bedalov and Simon, 2004). Increased SIRT1 activity in fibroblasts, and most likely neurons, have been shown to alter gene expression by targeting several transcription factors including p53 (Luo *et al.*, 2001; Vaziri *et al.*, 2001), forkhead-box (FOXO) transcription family (Brunet *et al.*, 2004; Motta *et al.*, 2004), and NF-κB (Yeung *et al.*, 2004). As SIRT1 activity responds to increased intracellular NAD^+ levels, it is possible that enhanced NAD^+ levels can induce several protective factors that will delay neuronal degeneration (Pallas *et al.*, 2008). On the other hand, impaired SIRT1 activity due to PARP mediated NAD^+ depletion can promote p53, FOXO and Bax activities which sensitise cells to apoptosis (Pillai *et al.*, 2005). Therefore, drugs that promote SIRT1 activity are highly likely to reduce further neurodegeneration in AD.

CONCLUSION

Oxygen free radical activity is responsible for important molecular cascades that underlie a number of pathologic processes including, neurodegeneration in AD, ischemia-reperfusion injury, atherosclerosis, inflammation and potentially tumour generation through deregulated cell signalling following DNA damage. Due to ongoing oxygen radical activity in the brain, vulnerable cells such as long-lived neurons require efficient DNA repair processes. An imbalance in favour of DNA damage will result in the activation of cellular apoptotic processes and progressive neuronal cell death as seen in AD.

Integral to DNA repair and cell survival after oxidative stress are the linked processes of NAD^+ dependent PARP and SIRT-1 activities and p53 function. Adequate cellular NAD^+ levels promote DNA repair, activate SIRT-1 linked deacetylation of histones and p53, promoting cellular metabolism and continuing viability. Maintaining adequate cellular NAD^+ levels under conditions of increased turnover during the aging process and in diseases such as AD identifies the NAD^+ synthetic pathway as a novel and potentially effective therapeutic target for oxidative stress-mediated CNS disorders.

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