Chapter 32

SAMP8: A model to understand the role of oxidative stress in age-related diseases including Alzheimer's disease

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Abstract

The senescence-accelerated mouse (SAM) is an accelerated aging model that was established through phenotypic selection from a common genetic pool of AKR/J mouse strain. Among the SAM mice, SAMP8 mice are considered as a good model for gerontological research as they exhibit learning and memory deficits with age. SAMP8 mice also show age-related increased oxidative stress, which correlated with increased expression of APP and consequently beta-amyloid (Aβ) protein, one of the components of senile plaques, a hallmark of Alzheimer's disease brain (AD). SAMP8 mice can, consequently, potentially serve as a good model to study the fundamental mechanisms of age-related learning and memory deficits including Alzheimer's disease. In this review, we discuss the SAMP8 mouse with respect to its role as a model to understand age-related disorders with special emphasis on oxidative stress.

Keywords: Senescence-accelerated mouse prone 8 (SAMP8); Oxidative stress; Proteomics; Antioxidant; Lipoic acid; N-acetyl cysteine

1. Introduction

The senescence-accelerated mouse (SAM) is an accelerated aging model that was established from the AKR/J strain [1]. There are nine major senescence-accelerated mouse prone (SAMP) substrains and three major senescence-accelerated mouse resistant (SAMR) substrains, each of which exhibits characteristic disorders [2]. Among these SAM mice SAMP8 mice is considered as a good model for gerontological research as they

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exhibit learning and memory deficits with increasing age. SAMP8 mice show age-related impairment in learning and memory [3] that occurs as early as at 2 months compared to SAMR1 mice of the same age. In addition SAMP8 mice also showed neuropathological changes such as reduction in spine density, astrogliosis, spheroidal axonal dystrophy etc. Further, SAMP8 mice also demonstrated increased deposition of beta-amyloid (Aβ) protein, one of the components of senile plaques, a hallmark of Alzheimer's disease (AD) brain, in various brain regions [2]. SAMP8 mice showed an age dependent increase in the levels of Aβ in the hippocampi of SAMP8 mice from 4 to 12 months of age [4] and studies from the same group also showed the presence of Aβ plaques in the hippocampus of a 21-month-old SAMP8 mouse [5,6]. Based on the above, SAMP8 may potentially serve as a good model to study the fundamental mechanisms of age-related learning and memory deficits including those related to AD. For details on the genotypes of SAMP please refer to Butterfield and Poon (2006) and Takeda et al. (2009) reviews [2,7]. In this current review, we discuss the SAMP8 mouse with respect to its role as a model of oxidative stress, especially with relevance to Alzheimer's disease.

2. Evidence of oxidative stress in SAMP8 mice

The free radical theory of aging hypothesizes that oxidative modifications by reactive oxygen species (ROS) on proteins, DNA, lipid membranes and other molecules cause cellular dysfunction and aging in humans and animals [8]. It is well known that free radical damage affects composition integrity of cell membranes, including neurons, and free radical damage has been implicated in the etiology of neurodegenerative diseases such as AD [9]. Numerous experimental evidences suggest that amyloid β-peptide (Aβ) has a central role in the pathogenesis of AD [10], and Aβ-associated oxidative stress induces damage to neurons *in vitro* [11,12] and *in vivo* [13–15]. The SAMP8 strain, which develops learning and memory deficits by 12 months of age [16,17] and has a shorter median lifespan than controls [18], presents a hippocampus which contains higher levels of both APP and soluble Aβ-peptide at 8 and 12 months of age than at 4 months [19]. Furthermore, Aβ-peptide has an amnestic effect when it is injected either into the hippocampus or the amygdala of SAMP8 mice [20]. This effect could be related to alteration of the fatty acid composition of neuronal membrane lipids leading to cell membrane destruction [16].

A higher oxidative state has been detected in various organs of SAMP strains compared with normal aging SAMR1 strains used as a control. The first data about oxidative stress markers in SAMP8 mice were obtained from brain. After a regional study, only the cerebral cortex but not other regions of the cerebrum showed a significant increased lipid peroxidation, protein carbonyl formation, and generation of reactive oxygen species in SAMP8 compared to the control SAMR1 strain [21]. Subsequently other research groups reported that the levels of lipid hydroperoxide and protein carbonyls increased in the brain, liver, lung, and heart of SAMP8 compared with SAMR1 [22–25]. Further studies conducted in brain and liver demonstrated that SAMP8 mice are exposed to elevated levels of oxidative stress since an early stage in their life (2 months of age) [22], and the increased oxidative stress in brain is a function of age [23,26,27]. In fact 12-month-old

SAMP8 mice had more oxidative stress than 4-month-old SAMP8 mice [26]. In contrast, Matsugo et al. [25] found that the lipid hydroperoxide level in brain did not show an age-dependent variation in SAMP8 and SAMR1 measured at different ages (3, 6, and 9 months of age) even if the brain level was higher in SAMP8 mice at all three ages compared to SAMR1. On the contrary, the lipid hydroperoxide level in peripheral organs increased with age [25].

Taken in total, these results support the hypothesis that oxidative stress in the brain and peripheral tissues such as liver, heart, and lung could lead to cognitive dysfunction and the senescence-related impairments and degeneration typical of SAMP8 mice.

3. SAMP8 and antioxidant enzymes

The severity of oxidative damage on brain membrane lipids depends both on the enzymatic and the non-enzymatic defense systems. The levels of antioxidants enzymes were found to be altered in AD brain compared to age-matched controls since the early stages of the disease [28,29]. This may contribute to increased production of free radicals and oxidative damage in AD brain.

Manganese superoxide dismutase (Mn-SOD) activity, an important antioxidant enzyme involved in detoxification of anion superoxide (O_2^-), is decreased in cerebral cortex of aged SAMP8 compared to SAMR1 control, suggesting that Mn-SOD inhibition may be involved in the increased oxidative stress in the SAMP8 mice brain [30]. Furthermore, the activity of glutamine synthase (GS), an oxidatively sensitive enzyme [31,32] involved in the conversion of glutamate to glutamine, is also decreased in aged SAMP8 mice when compared to young SAMP8 mice or age-matched SAMR1 mice [21,23]. Loss of GS activity may also contribute to excitotoxic neuronal death. Glutathione peroxidase (GPx) is another antioxidant enzyme involved in GSH-mediated cellular protection. Its enzymatic activity is significantly decreased in aged SAMP8 mice brain [33].

The activity of nitric oxide synthase (NOS), an important enzyme involved in NO production [34], is increased in aged SAMP8 [35]. In contrast, catalase activity in the cerebral cortex of SAMP8 is decreased, while the activity of acyl-CoA oxidase, a microperoxisomal H_2O_2-producing enzyme, is increased when compared to young SAMP8 mice and to age-matched SAMPR1 [21]. These results suggest that the abnormality of activities of these microperoxisomal enzymes may contribute to the early increase in oxidative stress observed in the cerebral cortex of SAMP8 mice [22]. An important consequence could be the imbalance between reactive oxygen species (ROS) production and induction of oxidative damage to proteins seen in the brain of SAMP8 [24,27,36]. This consideration is consistent with the notion that oxidative stress plays a significant role in the learning and memory deficits in SAMP8 mice [23].

4. SAMP8 and proteomics

One of the major effects of increased oxidative stress in brains is protein oxidation. Oxidized proteins may cause aggregation and/or inactivation [37,38]. Proteomic strategies based on 2-dimensional electrophoresis (2-DE) are powerful for quantitative and

qualitative analyses on specific tissues and cells. By proteomics analysis it is possible to investigate the expression of proteins and their oxidative modification in the brains from aged SAMP8 mice.

The expression of different proteins like neurofilament triplet L protein (NF-L), lactate dehydrogenase 2 (LDH-2), heat shock protein 86 (HSP86), and α-spectrin was found significantly decreased, whereas the expression of triosephosphate isomerase (TPI) was found significantly increased in the brain of aged SAMP8 mice [27] by proteomic analysis. Further, α-enolase, lactate dehydrogenase 2 (LDH-2), dihydropyrimidinase-like protein 2 (DRP-2), creatine kinase (CK), α-spectrin were significantly oxidized in old SAMP8 brains compared with the 4-month-old SAMP8. Oxidized proteins generally have lower activity [37,38]. Consequently, these oxidized proteins in aged SAMP8 mouse brain that are involved in energy metabolism, neuroprotection, and synaptic maintenance may impair these cellular functions. In fact, decreased energy metabolism, impaired neuroprotection, and synaptic dysfunction are all observed in aged SAMP8 mice brain [16,21,39–42].

Hippocampus, a critical brain region associated with cognitive decline during normal aging and various neurodegenerative diseases, showed a series of abnormalities in SAMP8. By proteomics analysis two proteins among the soluble hippocampal proteins were found markedly changed in SAMP8 mice as compared to SAMR1: Uchl3 and mitofilin [36]. Uchl3 (ubiquitin carboxyl-terminal hydrolase L3), involved in cytosolic proteolysis of oxidatively damaged proteins, was down-regulated. The decreased Uchl3 may implicate in the aggregation of oxidized proteins in the brain of SAMP8 mice. UCH is critical to the CREB phosphorylation-mediated long-term potential. The decrease of Uchl3 in SAMP8 may be involved in the CREB-mediated long-term potential and contribute to the cognitive deficits at an early age in SAMP8 compared to SAMR1. Mitofilin, one of the most abundant mitochondrial proteins, exhibited a basic shift of isoelectric point that may contribute to the mitochondrial dysfunction and age-associated neurodegeneration seen in SAMP8.

Another recent proteomic study demonstrated [43] that neurons and astrocytes isolated from SAMP8 mice compared to SAMR1 showed an altered proteome. Proteins that belong to cellular pathways of energy metabolism, biosynthesis, cell transduction and signaling, stress response, and the maintenance of cytoskeletal functions were identified. Most of the changes were cell-type specific. However, there was a general increase in cell transduction, signaling, and stress-related proteins and a decrease in cytoskeletal proteins in SAMP8 compared to SAMR1 mice brains. In addition, neurons showed an increased expression of proteins involved in biosynthetic pathways. Differences in neuronal and astrocytic proteomes indicated that both cell types are implicated in the brain protein pathway alterations of the SAMP8 mouse model of age-related neurodegeneration.

5. SAMP8 and antioxidant treatments

As noted above SAMP8 mice showed increased markers of oxidative stress and decreased antioxidant enzymes thereby favoring increased generation of ROS. Moreover, alterations

in mitochondrial energetics have been reported for these mice, which suggests that certain anti-oxidant and energy-protective strategies may prove therapeutic.

Lipoic acid (LA) has been shown to act as a powerful micronutrient with diverse pharmacologic and antioxidant properties [44]. In biological systems LA exists in proteins linked covalently to lysyl residues as a lipoamide. Lipoic acid exists in reduced and non-reduced forms, and the mitochondrial E3 enzyme, dihydrolipoyl dehydrogenase, reduces lipoate to dihydrolipoate at the expense of NADH. The antioxidant property of LA is associated with the reduced form of LA, dihydrolipoic acid that reacts with oxidants such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, singlet oxygen, and HNE. Further, the antioxidant property of the LA could be associated with the recycling of cellular antioxidants, including coenzyme Q (CoQ), vitamins C and E, and glutathione (GSH). Recent studies suggested that the LA antioxidant function occurs via its ability to function as a pro-oxidant and by activation of transcription factor Nrf2, which can lead to transcriptional activation of phase II detoxification enzymes as well as antioxidant proteins such as glutathione-*S*-transferases, gamma-glutamylcysteine synthetase, ferritin, NAD (P) H: quinone oxidoreductase-1, and heme oxygenase-1, etc. Hence, LA potentially could be useful as a treatment for a number of diseases that are associated with oxidative stress. Moreover, LA has been reported to readily cross the blood–brain barrier, and this would be helpful in diseases involving brain.

As mentioned above, SAMP8 mice overexpress APP and have elevated levels of Aβ in the brain and consequently have increased levels of beta-amyloid and free-radical associated damage to proteins and lipids [5,19]. Mitochondrial dysfunction has been reported in SAMP8 mice, which could be associated with the increased production of free radicals in these mice [45,46]. Further, SAMP8 mice also exhibit age-related impairment in memory and learning at age of 12-months [47]. LA treatment improves learning and memory in old-SAMP8 mice treated with LA as assessed by the T-maze footshock avoidance paradigm and lever press appetitive task. Further, LA treatment reduced age-associated increase in the markers of oxidative stress such as protein oxidation, lipid peroxidation, Electron Paramagnetic Resonance (EPR)-determined W/S ratio of a cysteine-selective spin label in brain membranes from old SAMP8 mice compared to young SAMP8 mice [26,48].

Proteomics studies from our laboratory showed that LA treatment significantly increased the expressions of three brain proteins, i.e., neurofilament triplet L protein, alpha-enolase, and ubiquitous mitochondrial creatine kinase, augmenting the expression of these proteins in LA treatment, suggesting that the administration of LA may improve cellular energetics and also improve the structure of neurons. Such changes could thereby play an important role in neuronal plasticity and consequently in improving the learning and memory process in old SAMP8 mice. LA treatment has also been shown to significantly decrease specific carbonyl levels of lactate dehydrogenase B, dihydropyrimidinase-like protein 2 (CRMP2), and alpha-enolase in the aged SAMP8 mice, and these proteins are important for cellular energetics and axonal growth [49]. The antioxidant effect of LA can also be related to its presence as a cofactor for a number of mitochondrial associated proteins such as alpha-ketoglutate dehydrogenase etc. [50].

Glutathione (GSH) is present in millimolar concentrations and is the most abundant antioxidant in the brain. In a reaction involving oxidative stressors, GSH is converted to oxidized glutathione (GSSG), and the ratio of GSH/GSSG is used as a measure to check the redox and oxidative stress status of the cell. The levels of GSH are maintained by GSH peroxidase and glutathione reductase activity. The reduced form of the GSH scavenges reactive oxygen species (ROS) and other toxic and highly reactive products of lipid peroxidation such as HNE and acrolein, in addition to its ability to protect the thiols groups in proteins. The nucleophilic adducts of HNE and acrolein by GSH are normally formed by the reaction of the enzyme glutathione-S-transferase (GST) and are effluxed out from the cell by the multidrug resistance protein-1 (MRP-1) [51,52]. Glutathionylation of proteins is a reversible process where the GSH is removed by the action of glutaredoxin, a thiol transferase [53], and this process might help to protect the proteins that are redox sensitive proteins. Previous studies from our laboratory have identified a number of proteins that showed glutathionylation in AD brain and this modification is further associated with decreased activity of the glutathionylated proteins [54,55]. Hence, one approach for potential therapy of age-related neurodegenerative disorders is to increase the levels of GSH in the cell to reduce the levels of oxidative stress and thereby prevent or delay the diseases associated with oxidative stress. The rate limiting amino acid for the synthesis of glutathione is cysteine. Since free cysteine is highly reactive, this amino acid is mostly present as a component in the non-protein form as GSH. Hence, to increase the levels of GSH a number of previous studies have used N-acetylcysteine, which has been shown to lead to increased levels of GSH in the brain and also peripheral cells [26,56]. Further, *in vitro* and *ex vivo* studies showed that intraperitoneal (i.p.) injection of NAC protected brain against peroxynitrite, hydroxyl radicals, and acrolein induced toxic effects [57–59]. Moreover, mice treated with NAC prior to intracerebroventricular (i.c.v.) injections of Aβ showed decreased oxidative stress markers and also had improved learning and memory [60]. The protective effect mediated by NAC has been reported to involve not just the elevation of GSH levels; rather, the NAC mediated protection also is via its effect on other signaling pathways including activation of the Ras/ERK pathway, stimulating p35/Cdk5 activity, and reduced phosphorylation/deactivation of MLK3-MKK7-JNK3 signaling cascade [61–63]. In SAMP8 mice NAC has been shown to partially restore memory deficits in SAMP8 aged mice as well as reducing levels of lipid peroxidation and protein carbonyls [26]. This reduced level of oxidative stress markers and improvement observed in learning and memory could be related to the antioxidant property associated with NAC via up-regulation of reduced glutathione levels or could be associated with the down-regulation of APP gene transcription [64].

As mentioned above, SAMP8 mice showed increased production of Aβ, and consequently the decrease in learning and memory in SAMP8 mice could be associated with Aβ-induced oxidative stress. To explore the role of Aβ in the SAMP8 induced learning and memory problem we have injected antisense in 12-month-old SAMP8 mice (antisense oligonucleotide targeted at the Aβ region of the APP gene), and showed that AO treatment improved learning and memory and also reduced oxidative stress [65]. Further using redox proteomics we found that the levels of the specific protein carbonyl levels of aldoase 3 (Aldo3), coronin 1a (Coro 1a) and peroxiredoxin 2 (Prdx2) were significantly

decreased in the brains of 12-month-old SAMP8 mice treated with AO compared to age-matched SAMP8 mice treated with random AO [66]. Oxidations of these proteins explain the increased observation of the markers of oxidative stress in these mice at 12-month of age and also loss of cellular energetics, all of which are important for learning and memory. Further, using expression proteomics we also showed that the protein level of α-ATP synthase was significantly decreased, whereas the expression of profilin (Pro-2) was significantly increased in the brains of SAMP8 mice treated with AO.

6. Future studies

This review summarizes the current findings of increased oxidative stress in SAMP8 mice and therapeutics approaches that were employed to protect against age-dependent increase in oxidative stress. Further studies are required to unravel the mechanisms of aging using this model. SAMP8 mice also show an abnormal APP and Aβ metabolism; hence, these mice could serve as a potential model for studying the role of APP in Alzheimer's disease pathogenesis. From this review, it is clear that oxidative stress is critically important for impairment of learning and memory and Aβ may play a significant role in age-related cognitive decline in SAMP8. SAMP8 mice could serve as a model to test the therapeutic efficacy of drugs used to treat age-associated diseases in which cognitive effects are clinically present.

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