Low-dose pterostilbene, but not resveratrol, is a potent neuromodulator in aging and Alzheimer's disease

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Abstract

Recent studies have implicated resveratrol and pterostilbene, a resveratrol derivative, in the protection against age-related diseases including Alzheimer's disease (AD). However, the mechanism for the favorable effects of resveratrol in the brain remains unclear and information about direct cross-comparisons between these analogs is rare. As such, the purpose of this study was to compare the effectiveness of diet-achievable supplementation of resveratrol to that of pterostilbene at improving functional deficits and AD pathology in the SAMP8 mouse, a model of accelerated aging that is increasingly being validated as a model of sporadic and age-related AD. Furthermore we sought to determine the mechanism of action responsible for functional improvements observed by studying cellular stress, inflammation, and pathology markers known to be altered in AD. Two months of pterostilbene diet but not resveratrol significantly improved radial arm water maze function in SAMP8 compared with control-fed animals. Neither resveratrol nor pterostilbene increased sirtuin 1 (SIRT1) expression or downstream markers of sirtuin 1 activation. Importantly, markers of cellular stress, inflammation, and AD pathology were positively modulated by pterostilbene but not resveratrol and were associated with upregulation of peroxisome proliferator-activated receptor (PPAR) alpha expression. Taken together our findings indicate that at equivalent and diet-achievable doses pterostilbene is a more potent modulator of cognition and cellular stress than resveratrol, likely driven by increased peroxisome proliferator-activated receptor alpha expression and increased lipophilicity due to substitution of hydroxy with methoxy group in pterostilbene.
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1. Introduction

Aging poses the greatest risk factor in the development of Alzheimer's disease (AD). With an ever-increasing population, AD incidence in the United States will jump from 4 million individuals currently affected with the disease to 14 million by 2050 (Larson et al., 1992). Of concern, despite valiant effort by the scientific field to understand the molecular underpinnings of this insidious disease, little progress has been made with regard to mechanisms, diagnostic tests, or treatments.

Research to identify mechanisms associated with AD and new therapies is currently being carried out in rodent models of AD. However, despite that 95% of AD cases are age-related, a mouse model of late-onset and/or age-related AD does not exist. Instead, current studies are carried out in mouse models which overexpress AD-related pathology

(amyloid-beta plaques and tau hyperphosphorylation inclusions [tangles]) associated with specific mutations present in early-onset AD (< 5% of total AD cases; Pallas et al., 2008a; Teruel, 2004). On the other hand, the SAMP8 model has many of the histopathological and behavioral indicators of AD (increased levels of oxidative stress [OS], hyperphosphorylation of tau, cognitive decline, amyloid-beta levels; Castillo et al., 2009; Pallas et al., 2008a). Importantly, this mouse is a model of accelerated aging, therefore it provides an excellent model to study the chronology of neurodegenerative changes associated with AD development and therapeutic opportunities from an aging perspective.

Over the years polyphenols, endogenously produced by plants as protection against predation, have been a source of interest due to their many beneficial effects on health and disease (Casadesus et al., 2004; Joseph et al., 2005). These biochemicals have shown numerous protective properties including antibiotic, anti-inflammatory, antioxidant, and anticarcinogenic amongst others, both in vivo and in vitro (Joseph et al., 2008; Rimando and Suh, 2008). One popular polyphenol is resveratrol, which is found in grapes and red wine has shown to have neuroprotective and cognitive enhancing properties (Bhavnani, 2003; Valenzano et al., 2006; Wang et al., 2006) and to induce apoptosis in cancer cells (Rimando and Suh, 2008), however only at high doses. In vitro, resveratrol is a potent activator of sirtuin 1 (SIRT1) (Borra et al., 2005; Howitz et al., 2003), thought to provide protection through downstream pathways including forkhead box (FOXO) proteins and manganese superoxide dismutase (MnSOD) modulation (Brookins Danz et al., 2009). In this context, increasing SIRT1 has been found to protect cells against amyloid-beta-induced reactive oxygen species (ROS) production and DNA damage, thereby reducing apoptotic death (Della-Morte et al., 2009; Kim et al., 2007). In vitro effects of resveratrol, through SIRT1 activation (Yeung et al., 2004), also include inhibition of proinflammatory nuclear factor-kappa B (NFκB) transcription (Holmes-McNary and Baldwin, 2000; Jang et al., 1997; Manna et al., 2000). Moreover, it has been demonstrated that AD neurons are rescued by the activation of SIRT1, through the administration of resveratrol (Camins et al., 2010; Della-Morte et al., 2009; Sun et al., 2010).

Pterostilbene is a phenolic compound chemically similar to resveratrol. Initially isolated from sandalwood, it is also found in fruits including grapes and blueberries, known for their beneficial effects on cognition and neuronal function during aging (Casadesus et al., 2004). Pterostilbene is a potent antioxidant and anti-inflammatory agent shown to have beneficial effects in the aging brain (Joseph et al., 2008; Remsberg et al., 2008; Rimando et al., 2002). Interestingly, in vitro, it has higher potency at inducing apoptosis in cancer cells than resveratrol (Mikstacka et al., 2007; Tolomeo et al., 2005), and shows powerful agonistic properties on the peroxisome proliferator-activated receptor (PPAR) alpha receptor (Rimando et al., 2005), a receptor complex that is intimately associated with fatty acid metabolism, inflammation, and oxidative stress regulation (Pyper et al., 2010).

To date, little is known about the biochemical and molecular mechanisms associated with pterostilbene's effects on neuronal function and cognitive function and whether this compound has protective effects in age-related pathological events. Given that the effects of resveratrol on neuronal function and SIRT1 activation have often been observed only when administered at high doses, the goal of this study was to (1) determine and evaluate the effectiveness of resveratrol at diet-achievable dose on cognition and neuronal function in a model of pathological aging and/or early AD while directly comparing it to pterostilbene; and (2) determine the mechanisms associated with the observed changes in both supplementation groups.

2. Methods

2.1. Animals and diet preparation

Five-month-old male and female SAMP8 were fed with either resveratrol or pterostilbene at an identical dose (120 mg/kg of diet) for 8 weeks or control diet, 120 mg/kg of diet equated to the content of resveratrol of in 2 glasses of wine. Animals were kept on a 12-hour light and 12-hour dark cycle with free access to food and water. Pterostilbene dose was kept identical to that of resveratrol to determine potency differences. In addition, an age-matched control SAMR1 group was included to be able to determine the magnitude of improvement produced by our experimental diets. Resveratrol (ChemPacific Corporation, Baltimore, MD, USA) and pterostilbene (synthesized according to Joseph et al., 2008), both nuclear magnetic resonance pure, were incorporated, separately, into Irradiated ProLab IsoPro RMH 3000 (TestDiet, Richmond, IN, USA). Compound incorporation was carried out by Harlan Teklad (Madison, WI, USA) at low drying temperature to prevent any degradation of the compounds. Body weight and diet consumption were tracked twice across the study to ensure that there were no diet intake related differences (i.e., diet taste preference).

2.2. Radial arm water maze

The radial arm water maze is a spatial learning and memory task that involves the use of distal visual cues to locate a hidden platform in 1 of 6 arms. Behavioral testing was carried out during the light cycle. Briefly, the test was carried out within a pool (120 cm diameter) with 6 swim arms; water temperature was kept constant at 24 °C for the duration of the testing sessions. One constant goal arm with a platform was used for the duration of the training and was randomized across animals to avoid spatial preference confounds. Animals were introduced into the water maze from different arms at every trial. On Day 1, 12 trials occurred (1-minute periods), alternating between a visible and hidden goal platform, with the exception of the last 3 trials where

the platform is hidden each time. Day 2 consisted of 12 trials using the hidden platform each time. Entering the nongoal arms during the trial was considered an error and was the dependent variable of this task. Successful learning of the task is considered 2 errors or less within the 1-minute trial. This particular protocol has been shown to be very sensitive in detecting spatial memory deficits in AD transgenic mice (Alamed et al., 2006). Data are reported as blocks of 3 trials for Day 1 (block 1–5) and Day 2 (block 6–10). Statistical analyses were carried out using a 1-way analysis of variance (ANOVA) followed by post hoc analysis using the Bonferroni multiple comparisons test.

2.3. Tissue processing

Animals were deeply anesthetized with a lethal dose of Avertin (10g tribromoethanol, 10mL tert amyl alcohol) (Acros Organics, Geel, Belgium) (500 mg/kg) and hippocampal and cortical tissue was collected and flash-frozen with liquid nitrogen and stored at -80 °C until homogenization. Samples were later lysed with 1x lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM Na_2 ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 1% triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na_3VO_4, 1 μg/mL leupeptin) (Cell Signaling, Beverly, MA, USA). Samples were centrifuged (10,000 rpm for 10 minutes) and the supernatant was collected. The protein concentration of the samples was determined by bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA).

2.4. Western blotting

Aliquots of tissue containing 20–40 μg of protein were loaded on 8%–10% acrylamide gels. Gels were then transferred to a polyvinylidenedifluoride membrane (Millipore, Billerica, MA, USA) overnight. Membranes were blocked in 10% nonfat dry milk in tris buffered saline (TBS), 0.1% Tween-20 for 1 hour at room temperature and probed with primary antibodies diluted in TBS, 0.1% Tween-20 overnight at 4 °C (see Supplementary Appendix S1 for antibodies and dilutions). After incubation with primary antibody (see Supplementary Table S1), membranes were washed (3 times for 10 minutes in TBS, 0.1% Tween-20) and probed with horseradish peroxidase (HRP) species-specific secondary antibodies in TBS, 0.1% Tween-20 for 1 hour at room temperature. Membranes were then washed (3 times for 10 minutes in TBS, 0.1% Tween-20) and bands were visualized using an ECL chemiluminescence-based detection kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA) on Kodak film (Kodak, Rochester, NY, USA).

2.5. Extraction of resveratrol and pterostilbene in serum

Serum was collected during sacrifice following standard procedures and kept in -80 °C until analysis. Serum samples were thawed in ice and two 150-μL aliquots were taken and transferred to 2 separate Eppendorf tubes. To 1 tube was added 60 μL of β-glucuronidase, 5000 U/mL potassium phosphate buffer (75 mM, pH 6.8 at 37 °C). To the other

tube, only potassium phosphate buffer (60 μL) was added. The samples were vortexed then incubated at 37 °C while shaking at 750 rpm for 20 hours. Thereafter, ice-cold High Performance Liquid Chromatography-grade acetonitrile was added in both tubes, vortexed, and centrifuged for 5 minutes at 5000 rpm, and 4 °C. The supernatant was collected, and dried under a stream of nitrogen for gas chromatography-mass spectrometry (GC-MS) analysis.

2.6. Extraction of resveratrol and pterostilbene in brain tissue

The brain tissues kept in -80 °C were thawed in ice. Three sample tissues were combined in an Eppendorf tube, and considered as 1 sample. To the tube was added 300 μL sodium phosphate buffer, and the tissues were homogenized manually for 2 minutes. Equal portions of the homogenates were transferred to 2 separate Eppendorf tubes. To 1 portion, 50 μL β-glucuronidase (5000 U/mL potassium phosphate buffer) was added. To the other portion 50 μL potassium phosphate buffer (without enzyme) was added. The samples were vortexed, and incubated (37 °C, 20 hours) while shaking (750 rpm). The samples were then centrifuged (15 min, 7000g, 4 °C). The supernatant was collected and partitioned with ethyl acetate (200 μL, twice). The ethyl acetate layers were combined, dried under a stream of nitrogen, and used for GC-MS analysis.

2.7. Quantification and analysis of Western blots

Western blots were quantified by densiometric measurements using Quantity One software Version 4.4 (Bio-Rad, Hercules, CA, USA) after appropriate background subtraction. All results are shown as mean average $\pm$ standard error of the mean. All results including behavioral data were analyzed with 1-way ANOVA and post hoc multiple comparisons to determine significant differences between groups at $p < 0.05$.

2.8. Analysis of serum and brain tissue by GC-MS

The nitrogen-dried samples were treated with 30 μL of a 1:1 mixture of N,O-*bis*[trimethylsilyl] trifluoroacetamide (BSTFA) and dimethylformamide (DMF) (both from Pierce Biotechnology, Inc., Rockford, IL, USA) and heated at 70 °C for 40 minutes. The derivatized samples were analyzed for levels of pterostilbene and resveratrol on a JEOL GCMate II Instrument (JEOL, USA Inc., Peabody, MA, USA) using a J&W DB-5 capillary column (0.25 mm internal diameter, 0.25 μm film thickness, and 30 mm length; Agilent Technologies, Foster City, CA, USA). The gas chromatography temperature program was: initial 190 °C held for 1 minute, increased to 244 °C at 30 °C per minute rate and held at this temperature for 0.5 minutes, increased to 246 °C at the rate of 0.2 °C per minute and held at this temperature for 0.5 minutes, then finally increased to 300 °C at the rate of 30 °C per minute and held at this temperature for 1 minute. The carrier gas was ultrahigh purity helium, at 1 mL per minute flow rate. The injection port was kept at

250 °C, the GC-MS interface at 230 °C, and the ionization chamber at 230 °C. The volume of injection was 2 µL (splitless injection). The mass spectrum was acquired in selected ion-monitoring mode, electron impact 70 eV. GC-MS analyses were in duplicates. The retention time of pterostilbene was 11.6 minutes, and resveratrol 13.7 minutes.

For the quantization of pterostilbene mass-to-charge ratio 328, 313, and 297 were used; for resveratrol mass-to-charge ratio 444, 429, and 341 were used. Quantitation was done using external standards of commercial samples of resveratrol (Sigma-Aldrich, St. Louis, MO, UA) and a synthetic sample of pterostilbene.

3. Results

3.1. Cognitive function

Analysis using a 1-way ANOVA with a repeated measures factor (day) indicated a significant difference in learning across groups ($p < 0.05$). Specifically, nontreated SAMP8 made significantly more errors in this task compared with pterostilbene-fed SAMP8 ($p < 0.05$) and SAMR1 controls ($p < 0.05$), suggesting that pterostilbene can normalize cognitive function to SAMR1 control levels. Interestingly, resveratrol-fed animals showed improved learning compared with nonfed SAMP8 animals but this difference was not statistically significant ($p = 0.07$) suggesting that pterostilbene is a more potent modulator of cognitive function compared with resveratrol (Fig 1). There were no significant changes in body weight and food intake between the groups.
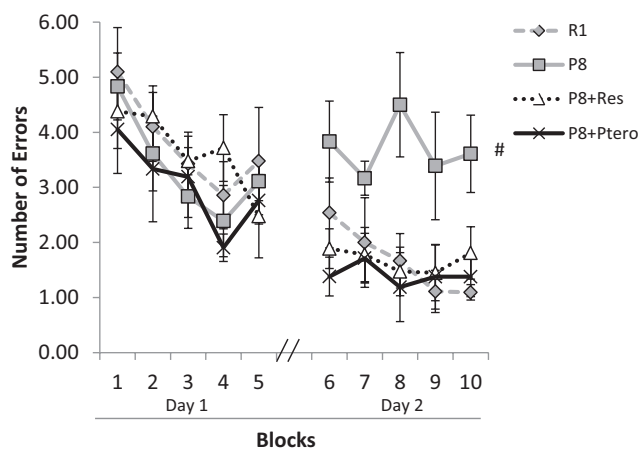

Fig. 1. Number of errors made over 2-day radial arm water maze test. SAMP8 mice were fed resveratrol, pterostilbene, and control diets. Statistical difference was found between groups by 1-way analysis of variance (ANOVA) with a repeated measures factor ($p < 0.05$). Post hoc comparisons between groups showed significant improvement in the SAMR1 ($n = 9$) and pterostilbene groups ($n = 6$) from the SAMP8 group ($n = 8$) ($^\# = p < 0.05$). Resveratrol ($n = 6$) was not significantly improved compared with SAMP8 but was not significantly different from SAMR1 and pterostilbene groups.

3.2. Bioavailability of pterostilbene and resveratrol

To test the bioavailability of pterostilbene in comparison with resveratrol, we measured the levels of both compounds in the serum as well as brain tissue homogenate. Previous studies have shown that pterostilbene shows a much higher bioavailability than resveratrol (Kapetanovic et al., 2011). Supporting these results, our data shows that pterostilbene was more abundant in the serum compared with resveratrol. Importantly, we were able to detect pterostilbene in brain tissue homogenate but not resveratrol (Supplementary Fig. S1).

3.3. Activation of SIRT1 and downstream targets

In order to determine the ability of dietary achievable doses of pterostilbene and resveratrol to modulate makers previously indicated to be significantly increased by resveratrol, we determined the levels of SIRT1 expression in all 4 groups. Our data indicate that SIRT1 expression was not significantly different between groups (Fig. 2B). In order to verify our SIRT1 results we also measured acetylation of p53, known to be inhibited by SIRT1 (Solomon et al., 2006). Our data show that acetylated p53 was not significantly changed by any of our treatments ($p = 0.19$) (Fig. 2C).

3.4. Upregulation of MnSOD through pterostilbene

Both pterostilbene and resveratrol have shown antioxidant properties in vivo and/or in vitro (Mikstacka et al., 2007). Furthermore, MnSOD expression has been shown to be increased by resveratrol at higher doses (Brookins Danz et al., 2009). To address the comparative effectiveness of these 2 chemically similar compounds in addition to determining their effectiveness at dietary-achievable doses, we measured changes in MnSOD expression. The groups were found significantly different through ANOVA ($F = 8.258$; $p < 0.01$). Our findings indicated that MnSOD levels significantly decreased in the SAMP8 group compared with the SAMR1 control group ($p < 0.01$) (Fig. 3C). Notably, pterostilbene rescued the levels of MnSOD back to the SAMR1 levels, an effect that was absent in the resveratrol-fed group.

3.5. PPAR-α rescue

Peroxisome proliferator-activated receptor alpha have been found to increase levels of MnSOD levels in the brain (Wang et al., 2010). Therefore, given in vitro data suggesting that pterostilbene is a potent PPAR-α agonist, we sought to determine whether changes in MnSOD observed in the pterostilbene-treated animals were due to upregulation of PPAR-α. Our results indicated a significant difference across groups ($F = 4.534$; $p < 0.05$) (Fig. 3B). Post hoc analysis revealed that PPAR-α is significantly decreased in the SAMP8 group compared with the SAMR1 control group ($p < 0.05$). Importantly, while resveratrol treatment had no
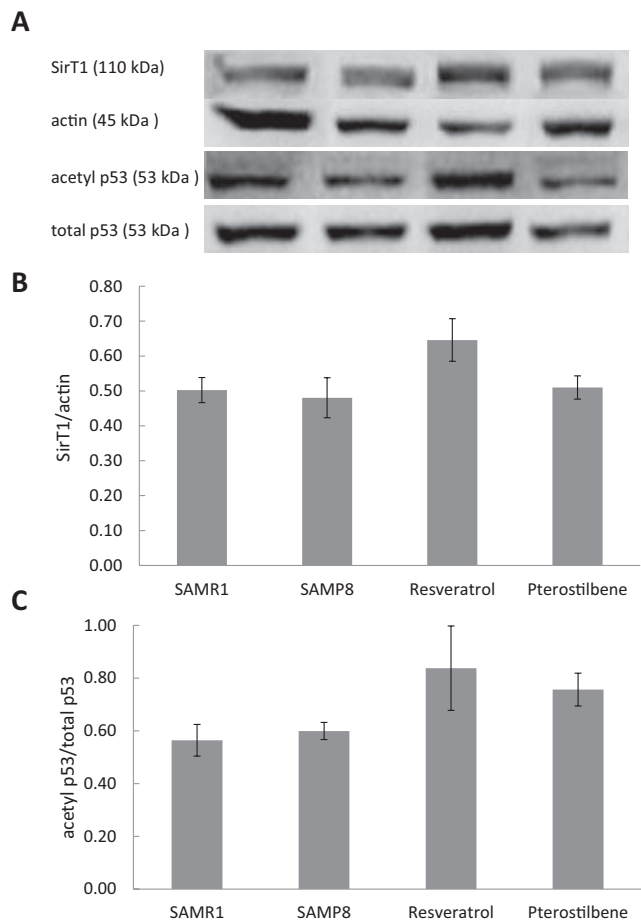
A

B

C

Fig. 2. (A) Western blot of sirtuin 1 (SIRT1) (with β-actin control) and acetyl p53 expression (with total p53 control) in mouse hippocampus. (B) SIRT1/actin ratio. Results are representative of protein expression from each group. No statistical difference was found between groups by 1-way analysis of variance (ANOVA). (C) Acetyl p53/total p53 ratio. No statistical difference was found between groups by 1-way ANOVA.

significant effect on PPAR-α expression, pterostilbene rescued PPAR-α expression to SAMR1 control levels.

3.6. NF$\kappa\beta$ activation

To further confirm the PPAR alpha changes observed in the SAMP8 mouse and the effectiveness of pterostilbene in normalizing these effects, we measured NF$\kappa\beta$, also known to be inhibited by PPAR α activation (Nunn et al., 2007). NF$\kappa\beta$ p65 level comparisons across groups revealed a significant group difference ($F = 4.534$; $p < 0.05$). Post hoc analyses indicated that NF$\kappa\beta$ p65 levels in SAMP8 mice were significantly higher than those in the control SAMR1 group ($p < 0.01$). This increased expression was not rescued by resveratrol treatment indicated by a significant increase of NF$\kappa\beta$ p65 levels in the resveratrol-treated group compared with the SAMR1 control group ($p < 0.05$). Pterostilbene treatment did not inhibit NF$\kappa\beta$ p65 levels significantly compared with SAMP8 but showed a strong trend toward significance

($p = 0.06$). Moreover, pterostilbene was not statistically different from SAMR1 control levels indicating at least a partial rescue by pterostilbene dietary supplementation (Fig. 3D).
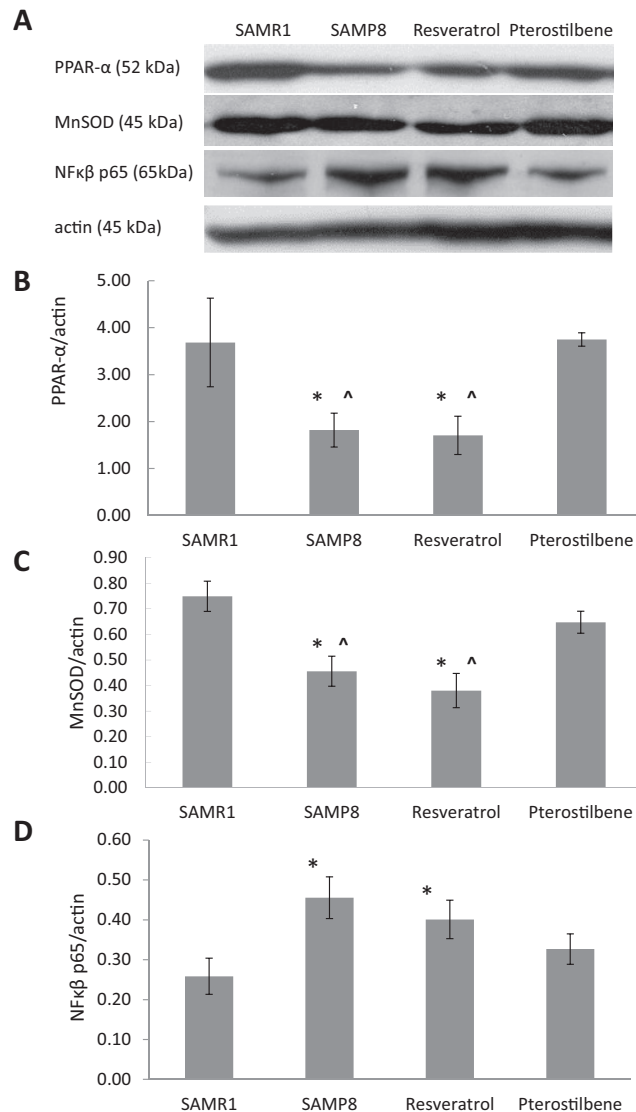
A

B

C

D

Fig. 3. (A) Western blot of peroxisome proliferator-activated receptor (PPAR)-α, manganese superoxide dismutase (MnSOD), and nuclear factor-kappa B (NFκB) p65 (with β-actin control) in mouse hippocampus. (B) PPAR-α/actin ratio. Results are representative of protein expression from each group. Groups were found to be statistical different by 1-way analysis of variance (ANOVA) ($p < 0.05$). Post hoc comparisons showed significant decrease in SAMP8 ($n = 8$) compared with SAMR1 ($n = 9$) (* = $p < 0.05$) and was reversed by pterostilbene treatment ($n = 6$) (^ = $p < 0.05$) but not resveratrol ($n = 6$). (C) MnSOD/actin ratio. Statistical significance by ANOVA ($p < 0.05$). Post hoc comparisons showed significant decrease in SAMP8 compared with SAMR1 (* = $p < 0.05$) and was increased by pterostilbene treatment (^ = $p < 0.05$). (D) NF$\kappa\beta$ p65/actin ratio. Statistical significance found by ANOVA ($p < 0.05$). Post hoc comparisons showed a significant increase in NF$\kappa\beta$ p65 levels in both SAMP8 and resveratrol-fed groups in comparison with SAMR1 control (* = $p < 0.05$).

3.7. Decreased levels of phosphorylated JnK

One signaling network dedicated to cellular maintenance under stress conditions involves stress-activated protein kinases (SAPKs), also known as Jun NH2-terminal kinases (JNKs). Increased JNK phosphorylation has been intimately associated with oxidative stress and inflammatory processes (Joseph et al., 2010; Liu et al., 2010) and well described in AD (Zhu et al., 2001a, 2001b). In this regard, we found a significant difference across groups ($F = 6.89$; $p < 0.01$) (Fig. 4B). We determined that SAMP8 had a high phosphorylation of JNK in comparison with SAMR1 control group ($p < 0.05$). Resveratrol-fed animals did not show a reduction in JNK phosphorylation and remained significantly elevated compared with SAMR1 controls. Importantly, pterostilbene-fed animals showed a reduction in JNK phosphorylation levels compared with SAMP8 groups ($p < 0.05$), similar to levels of SAMR1 control animals.

3.8. Reduced phosphorylation of tau (PHF)

Hyperphosphorylation of tau is intimately associated with cellular stress processes including JNK phosphorylation (Melov et al., 2007; Su et al., 2010; Zhu et al., 2002). We found a group difference in tau phosphorylation levels ($F = 3.318$; $p < 0.05$). Specifically, SAMP8 nontreated animals were found to have a significantly higher level of PHF-1 expression compared with the SAMR1 control group ($p < 0.05$) (Fig. 4C). Resveratrol was not effective at downregulating PHF-1 expression but pterostilbene supplementation was able to restore levels to SAMR1 controls.

4. Discussion

Alzheimer's disease (AD) poses an ever increasing threat with an aging population. SAMP8 mice have many of the histopathologic and behavior markers of AD including cognitive decline. It has been shown in several studies that many polyphenols such as resveratrol have antioxidant properties that may help the degradation that occurs with AD and modulate cascades associated with aging (Brisdelli et al., 2009). However, these studies often use doses not achievable through the diet. In our study we show that dietary supplementation of pterostilbene or resveratrol improved cognitive function in the SAMP8 model. Nevertheless, pterostilbene showed significant improvement over resveratrol. Furthermore, pterostilbene was more potent in activating protective signaling cascades and downregulating stress cascades at doses same as resveratrol, independent of SIRT1 regulation. This was shown by the lack of differences across groups in SIRT1 expression or its downstream targets such as acetylated p53 (Solomon et al., 2006). Importantly, the fact that resveratrol tended to increase both SIRT1 and acetylated p53 but was unable to upregulate more indirect targets such as acetylation of p53 suggests that while active, diet achievable doses were not sufficient to drive robust SIRT1 signaling. Previous studies have
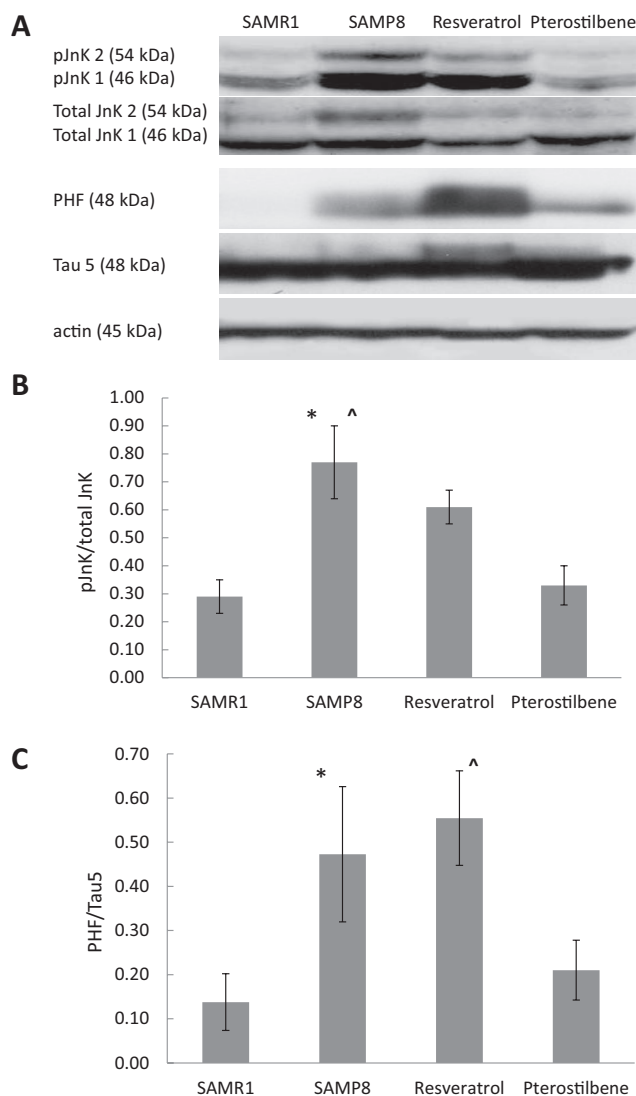

Fig. 4. (A) Western blot of phosphorylated jun NH2-terminal kinases (pJNK) (with total JNK control) and phosphorylation of tau (PHF) (with Tau5 control) in mouse hippocampus. (B) pJNK/total JNK ratio. Results are representative of protein expression from each group. Statistical difference was found between groups by 1-way analysis of variance (ANOVA) ($p < 0.05$). Post hoc comparisons between groups showed significant difference SAMP8 ($n = 8$) group and SAMR1 ($n = 9$) control (* = $p < 0.05$) and resveratrol ($n = 6$) and pterostilbene ($n = 6$) groups (^ = $p < 0.05$). (C) PHF/tau 5 ratio. Statistical difference was found between groups by 1-way ANOVA ($p < 0.05$). Post hoc comparisons between groups showed significant difference between SAMP8 group and SAMR1 control (* = $p < 0.05$) and resveratrol and pterostilbene groups (^ = $p < 0.05$).

shown that SIRT1 expression is decreased in the SAMP8 mouse compared with SAMR1 controls (Pallas et al., 2008b). However, analyses were carried out in older animals suggesting that the lack of differences between R1 and P8 mice observed in our study may be due to the age of the experimental groups.

Previously, studies have shown that cognitive improvements are likely not to be fully dependent on SIRT1 (Julien et al., 2009; Kim et al., 2007). Therefore we looked into

downstream mechanisms associated with aging and AD and shown to be important to neuronal and cognitive function, such as oxidative stress and inflammation (Castellani et al., 2008, 2009; Joseph et al., 2007; Shukitt-Hale et al., 2008, 2009). In this regard, our findings demonstrate that SAMP8 mice fed the control diet show low levels of MnSOD, an endogenous antioxidant defense, compared with SAMR1 controls and that these decreases in MnSOD was reversed only by pterostilbene. This supports previous data demonstrating powerful antioxidant effects of pterostilbene (Mikstacka et al., 2010). Here we show that, at least partially, these antioxidant effects are driven through the upregulation of endogenous antioxidant systems. Interestingly, resveratrol was not able to increase MnSOD activity in our study. Previous data demonstrate the ability of resveratrol to modulate MnSOD expression via the SIRT1/FOXO pathway both in vitro (Danz et al., 2009) and in vivo (Kao et al., 2010; Pfluger et al., 2008), however, at high doses. Furthermore, the fact that MnSOD was upregulated by pterostilbene in the absence of changes in the expression of SIRT1, suggests that the effects of pterostilbene on MnSOD were driven through an alternative mechanism.

One modulator of MnSOD is PPAR alpha (Ding et al., 2007). Importantly, PPAR alpha agonists have been shown to be neuroprotective after stroke (Ouk et al., 2009) and to increase hippocampal neurogenesis, a molecular event associated with cognition (Ramanan et al., 2009). Given that pterostilbene has been shown to be a powerful agonist of the PPAR alpha receptor in vitro (Rimando et al., 2005), we measured changes in PPAR alpha protein expression in our groups. Our findings demonstrate that PPAR alpha protein expression is downregulated in the SAMP8 mouse and is normalized by pterostilbene. Furthermore, it has been shown that ligand binding to the PPAR alpha stabilizes it by decreasing ubiquitinization and degradation (Blanquart et al., 2002). Therefore, 1 possibility is that pterostilbene, through its potent PPAR agonist properties, decreases the degradation of the protein resulting in high protein expression of PPAR alpha observed in our study.

To further elucidate the relationship between PPAR alpha modulation by pterostilbene we determined the ability of this stilbenoid to modulate NF$\kappa\beta$ expression. PPAR alpha has been shown to form physical inactive complexes with NF$\kappa\beta$ p65 hence reducing the ability to drive transcriptional activity (Delerive et al., 1999; Dragomir et al., 2006; Nunn et al., 2007). Our data demonstrates that SAMP8 mice show increases in NF$\kappa\beta$ expression compared with SAMR1 controls and pterostilbene treatment, but not resveratrol, is able to normalize these levels, consistent with upregulation of PPAR alpha activation.

Oxidative stress is a well established pathogenic factor in aging and AD (Markesbery, 1997; Perry et al., 1998; Smith et al., 1995b) and the association of oxidative stress with tau abnormalities is well known (Calingasan et al., 1999a, 1999b; Smith et al., 1995a; Takeda et al., 2000). These changes are also observed in the normal aging process as well as along the length of the axon (Wataya et al., 2002) suggesting that the oxidative modification of cytoskeletal proteins is under tight regulation. Interestingly, both tau and neurofilament protein appear uniquely adapted to oxidative attack due to their high content of lysine-serine-proline (KSP) domains and exposure of these domains on the protein surface is affected by extensive phosphorylation of serine residues resulting in an oxidative sponge of surface-modifiable lysine residues (Wataya et al., 2002). Because phosphorylation plays this pivotal role in redox balance, it is perhaps not surprising that oxidative stress, through activation of various cell signaling pathways including SAPK/JNK, leads to phosphorylation of tau (Ekinci and Shea, 2000a, 2000b; Joseph et al., 2010; Ramiro-Puig et al., 2009; Zhu et al., 2000, 2001a). Also of note is the fact that inhibition of JNK phosphorylation can be modulated by PPAR alpha agonists (Martínez de Ubago et al., 2009) and has also been shown to repress cognitive decline (Waetzig and Herdegen, 2005). Here we showed that phosphorylated JNK was significantly higher in SAMP8 mice compared with SAMR1 controls, an increase that was rescued by pterostilbene but not resveratrol. Importantly, tau phosphorylation pathology showed a similar trend. As previously reported in older mice, SAMP8 mice show increased phosphorylation of tau in comparison with the SAMR1 controls (Tajes et al., 2008; Tomobe and Nomura, 2009). In our study we show that increased tau phosphorylation was rescued by pterostilbene but not resveratrol.

In summary, while resveratrol shows important protective effects these may be restricted to when given at higher doses (Bhavnani, 2003; Sönmez et al., 2007). Our data demonstrate that pterostilbene is a more potent effector of beneficial molecular and functional events than resveratrol in the SAMP8 mouse. Importantly, these benefits are independent of SIRT1 activation and are likely driven through PPAR alpha regulation known to influence MnSOD expression (Ding et al., 2007), NF$\kappa\beta$ transcription (Delerive et al., 1999; Dragomir et al., 2006; Nunn et al., 2007), and JNK phosphorylation (Martínez de Ubago et al., 2009), all shown to be significantly improved by pterostilbene. In turn, all of these events are known to modulate tau pathology, as such it is not surprising that tau phosphorylation at sites associated with AD pathology were downregulated by pterostilbene. One potential explanation that can account for our findings is related to the chemical structure differences between these 2 compounds. In this regard, the substitution of the hydroxy group of resveratrol with a methoxy group in pterostilbene makes this molecule more lipophilic (Cichocki et al., 2008). This change may lead to better bioavailability of pterostilbene and consequently a more potent neuroprotective effect in the brain. This is supported by the fact that while diet consumption and weights did not vary in our studies, pterostilbene was found at higher doses both in serum and brain tissue compared with resveratrol, which

was found at low levels in the serum and undetectable in the brain tissue. While it is yet to be determined whether the cognitive improvements induced by pterostilbene in the SAMP8 model can be applied to humans, recent reports demonstrate that fruits containing pterostilbene such as blueberries ameliorate cognitive function in aged humans (Joseph et al., 2008) and that PPAR alpha agonists afford central nervous system protection (Hanyu et al., 2010). Therefore, use of pterostilbene may become an effective, natural, therapeutic strategy to improve cognitive function in aging and potentially a strategy to slow down the development of AD.

Disclosure statement

Drs. Casadesus, Lee, Camins, Pallas, Rimando, and Shukitt-Hale have no conflict of interest or disclosures to provide. Dr. Smith and Dr. Joseph recently passed away. Dr. Joseph had no conflict of interest or disclosures and Dr. Mark Smith was a consultant for Anavex Life Sciences Corporation, Eisai, Medivation, Neurotez, and Takeda Pharmaceuticals; owned stock options in Aria Neurosciences, Neurotez, Panancea, and Voyager, and received lecture fees from GSK, Medivation, and Pfizer. Dr. Zhu was also a consultant for Medivation. None of these companies are directly or peripherally implicated in the work hereby submitted. Jaewon Chang and David Porquet are students with no conflicts or disclosures to provide.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2011.08.015.

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