



SIRT2 and FOXO3a expressions in the cerebral cortex and hippocampus of young and aged male rats: antioxidant and anti-apoptotic effects of melatonin

Arzu Keskin-Aktan¹ · Kazime Gonca Akbulut² · Samira Abdi³ · Hakan Akbulut³

Received: 29 October 2020 / Accepted: 17 October 2021 / Published online: 27 October 2021
© Akadémiai Kiadó Zrt. 2021

Abstract

Melatonin has antioxidant, anti-apoptotic and anti-aging effects in the brain. Sirtuin2 (SIRT2) accumulates in the central nervous system with aging, and its inhibition appears to be protective in aging and aging-related neurodegenerative diseases. Forkhead Box-class O3a (FOXO3a) transcription factor is one of the main targets of SIRT2, and SIRT2-mediated FOXO3a deacetylation is closely related to aging, oxidative stress, and apoptosis. This study aimed to investigate the effects of melatonin on SIRT2 and FOXO3a expressions in the cerebral cortex and hippocampus of aged rats. Young (3 months, $n = 18$) and aged (22 months, $n = 18$) male Wistar rats were divided into control (4% DMSO-PBS, sc, for 21 days), melatonin (10 mg/kg, sc, for 21 days) and salermide (1 mM; 25 μ l/100 g bw, ip, for 21 days) groups. SIRT2, FOXO3a, Bcl-2, Bax and Bim expressions in the cerebral cortex and hippocampus were demonstrated by Western blotting. SIRT2 and FOXO3a protein levels were also measured by a sandwich ELISA method. Oxidative stress index (OSI) was calculated by measuring total oxidant status (TOS) and total antioxidant status (TAS). Aging increased SIRT2, FOXO3a, Bim (only in the cerebral cortex), Bax (only in the hippocampus), TOS, and OSI, while decreasing Bcl-2, Bcl-2/Bax and TAS in both brain regions. Melatonin decreased SIRT2, FOXO3a, oxidative stress parameters and pro-apoptotic proteins, while increasing TAS, Bcl-2 and Bcl-2/Bax, more specifically in the hippocampus of the aged brain. Our results indicate that inhibition of SIRT2 and FOXO3a expressions appears to be involved in the protective effects of melatonin in the hippocampus of aged rats.

Keywords Aging · Oxidative stress · Sirtuin 2 · FOXO3a · Melatonin · Brain

Introduction

Brain aging is associated with many neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and Huntington's disease (HD) and is critical in the development of new treatment targets. The neocortex and hippocampus are highly sensitive to oxidative stress, inflammation, and metabolic changes, and therefore, these two brain regions are the

first place where neurodegenerative changes are observed (Clarke et al. 2018; Fjell et al. 2014; Xinkun Wang and Michaelis 2010). It is known that pathological processes in the aging brain are related to the sirtuin and FOXO family, which are effective on life span, metabolism, apoptosis and oxidative stress (Jenwitheesuk et al. 2018).

Sirtuins are the members of the nicotinamide adenine diphosphate (NAD⁺)-dependent histone/protein deacetylase family, which can extend life through by maintaining homeostasis and genome stability (Michan and Sinclair 2007). SIRT2 is a member of the Sirtuin family (SIRT1–SIRT7). SIRT2, which is abundantly expressed in the brain, increases in response to oxidative stress, DNA damage and aging (Anwar et al. 2016; Braidy et al. 2015; Keskin-Aktan et al. 2018; Maxwell et al. 2011).

FOXO family members (FOXO1, FOXO3a, FOXO4, FOXO6) in mammals are involved in the insulin/IGF (insulin-like growth factor) pathway that regulates metabolism, apoptosis and aging. The highest mRNA expression level

✉ Arzu Keskin-Aktan
arzu.aktan@afsu.edu.tr

¹ Department of Physiology, School of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar 03200, Turkey

² Department of Physiology, School of Medicine, Gazi University, Ankara, Turkey

³ Department of Basic Oncology, Institute of Cancer Research, Ankara University, Ankara, Turkey

of FOXO1 is observed in fat tissue, FOXO3 in the brain, FOXO4 in the heart and FOXO6 in the developing brain (Greer and Brunet 2005).

One of the main targets of SIRT2 is the FOXO3a (Forkhead Box, class O3a) transcription factor, and SIRT2-mediated FOXO3a deacetylation is required to cope with oxidative stress by increasing the antioxidant enzymes such as manganese-SOD and catalase. However, activation of SIRT2 induces apoptosis by deacetylating FOXO3a and stimulating the translocation of FOXO3a to the nucleus (Wang et al. 2007). An increase in FOXO3a activity causes neurodegenerative diseases such as AD and PD (Pino et al. 2014; Qin et al. 2008). FOXO3a expression in the aging brain is not clear yet. Increased FOXO3a expression with aging has been reported in the occipital lobe (Braidly et al. 2015) and hippocampus (Jackson et al. 2009). However, FOXO3 expression has also been reported to decrease with aging (Huang et al. 2011; Park et al. 2011).

The brain is highly susceptible to oxidative stress. While the products of lipid peroxidation (malondialdehyde), protein oxidation and DNA oxidation (8-hydroxydeoxyguanine) increase in the brain with aging, antioxidant enzymes such as glutathione (GSH), GSH-reductase, GSH-S transferase, thioredoxin reductase and superoxide dismutase (SOD) decrease. This pattern may also manifest itself as an increase in total oxidant status and a decrease in total antioxidant status in tissues (Akbulut et al. 2008; Öztürk et al. 2012; Pollack et al. 2002). Increased neural losses are observed with oxidative stress in the aging brain. There is mutual interaction between family members of Bcl-2 (B-cell lymphoma/leukemia-2) in the induction or suppression of apoptosis. Bcl-2 inhibition and/or the activation of pro-apoptotic proteins such as Bax, Bak and Bim induced apoptosis in the neurons (Becker et al. 2004; Caballero et al. 2009; Xu et al. 2007). Among the Bcl-2 family members working at critical checkpoints of apoptosis, Bcl-2, Bcl-xL, Bcl-w and Mcl-1 anti-apoptotic, Bax, Bak, Bid, Bim, Puma, Noxa and Bcl-xs have pro-apoptotic effects.

Pharmacological (sirtinol, salermide, AGK-2, AK-7, etc.) or genetic inhibition of SIRT2 appears to be protective in neurodegenerative diseases such as AD (Biella et al. 2016), PD (Chen et al. 2015) and HD (Luthi-Carter et al. 2010). Salermide *N*-{3-[(2-hydroxy-naphthalene-1-ylmethylene)-amino]-phenyl}-2-phenyl-propionamide, used as a pharmacological SIRT2 inhibitor in the present study, can inhibit SIRT2 activity by 80% *in vitro*, and it is well tolerated by mice at concentrations up to 100 μ M (Alcaín and Villalba, 2009). Salermide increases acetylated p53 and apoptosis *in vitro* cancer cells (Peck et al. 2010) but not in fibroblasts (Lara et al. 2009).

Melatonin, the main hormone of the pineal gland, is an endogenous free radical scavenger and antioxidant (Tan et al. 1993). Melatonin easily crosses the blood–brain barrier, and

its receptors have a widespread distribution in the brain. Melatonin administration suppresses increased inflammation, oxidative stress and apoptotic activity in the aged brain, which result in increased survival rates of neurons (Akbulut et al. 2008; Caballero et al. 2009; Kireev et al. 2013). Melatonin decreases with age, while SIRT2 increases. In the present study, we aimed to investigate the effects of melatonin treatment or SIRT2 inhibition on oxidative stress, apoptosis, SIRT2 and FOXO3a expression in two important regions of the brain, such as the cerebral cortex and hippocampus, and the role of Sirtuin and FOXO families in becoming a new treatment target in brain aging and aging-related neurodegenerative diseases.

Materials and method

Animals and treatments

A total of 36 Wistar albino male rats were provided by Gazi University Laboratory Animal Breeding and Experimental Research Center (GUDAM). All rats were kept under standard laboratory conditions with equal periods of light and dark (12 h light/12 h dark; 08:00/20:00) at 23 °C and were fed commercial rat chow and water *ad libitum*. Animal treatments and all the methods used in the present study were approved by the Animal Experiments Ethics Committee of Gazi University (animal study protocol number: #G.U.ET-16.008).

Young (3 months, $n = 18$) and aged (22 months $n = 18$) male rats were divided into six groups: Young Control ($n = 6$), Young-Melatonin ($n = 6$), Young-Salermide ($n = 6$), Aged Control ($n = 6$), Aged-Melatonin ($n = 6$) and Aged-Salermide ($n = 6$) (Fig. 1). Melatonin (M5250-1G, Sigma-Aldrich) was dissolved in ethanol then diluted with phosphate buffer (1% ethanol-PBS, pH 7.4). Salermide (13,178, Cayman Chemical) was dissolved in dimethyl sulfoxide (DMSO) then diluted with PBS (4% DMSO-PBS). In our previous study, we found that there was no significant difference in the parameters investigated (SIRT2 activity, oxidative stress and apoptosis) between the groups treated with 1% ethanol-PBS or 4% DMSO-PBS. Daily treatments of melatonin (10 mg/kg, 100 μ l/100 g bw, sc), salermide (1 mM; 25 μ l/100 g bw, ip) and control groups (4% DMSO-PBS, sc) were administered as described in our previous studies (Akbulut et al. 2008; Keskin-Aktan et al. 2018). All injections were continued for 21 days, at 17:00. On the 22nd day of the experiment, the rats were sacrificed by taking intracardiac blood under ketamine-xylazine anesthesia. The cerebral cortex and hippocampus were isolated according to the atlas of Paxinos and Watson, and tissues were stored at -80 °C.

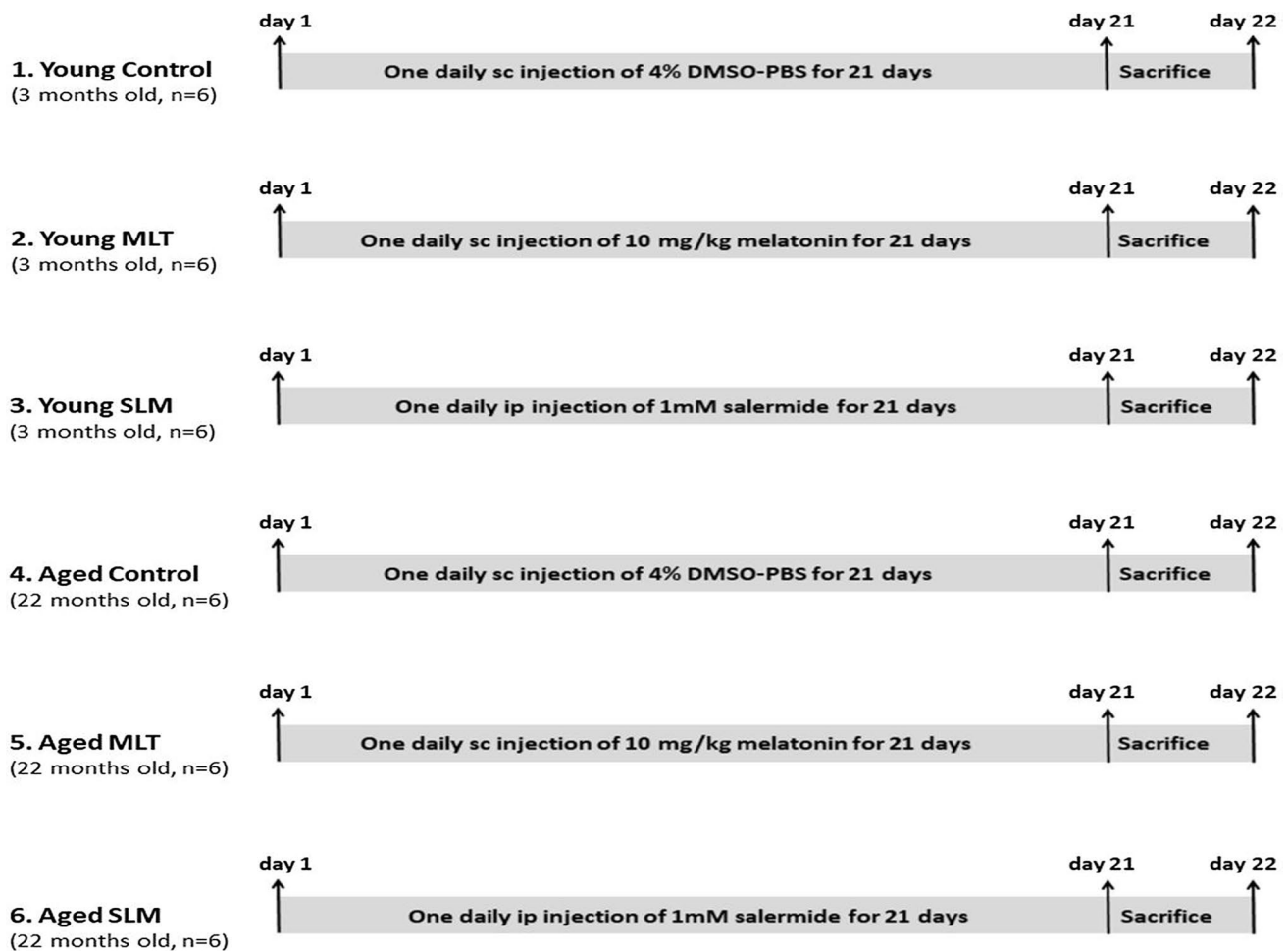


Fig. 1 The schematic of the experimental design and timeline of drug treatment of each group

Measurement of total oxidant status (TOS) and total antioxidant status (TAS)

The cerebral cortex and hippocampus tissues were homogenized by sonication in 140 mM KCl buffer (1:9, w/v, pH 7.4). Tissue homogenates were centrifuged at 3,000 rpm, + 4 °C for 5 min, and the supernatants were stored at –80 °C. Commercially available kits for TOS and TAS (RL0024, RL0017, Rel Assay Diagnostics) were used as recommended. Spectrophotometric readings were performed at 595 nm (for TOS) and 660 nm (for TAS). The assay was calibrated with H₂O₂ (10 μmol/L), and the results for TOS were expressed in μmol H₂O₂ Equiv./L. The assay was calibrated with Trolox Equivalent (1 mmol/L), a stable antioxidant standard solution, and the results for TAS were expressed in mmol Trolox Equiv./L.

Oxidative stress index (OSI)

Percent ratios of TOS to TAS level were accepted as OSI, which is considered an indicator of oxidative stress level. For the calculation of OSI, TAS unit (mmol Trolox Equiv./L) was changed to μmol Trolox Equiv./L, and OSI value was calculated according to the following formula: [(TOS, μmol H₂O₂ Equiv./L)/(TAS, μmol Trolox Equiv./L) × 100] (Yanik et al. 2004).

Western blot analysis

The cerebral cortex and hippocampus tissue lysates were prepared on ice with RIPA Lysis Buffer (pH 7.4) (Santa Cruz Biotechnology). 300 μl of lysis buffer for 20 mg of tissue containing phenylmethanesulfonyl fluoride (200 mM), sodium orthovanadate (100 mM) and protease inhibitor

cocktail. The lysates were centrifuged twice at 10,000g, 10 min at +4 °C, after 30 min incubation on ice. Proteins were quantified using Bradford's method (Bradford, 1976), and 20 µg of protein (40 µg for FOXO3a) was separated with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10%). Blotted nitrocellulose membranes (Bio-Rad) were blocked with freshly prepared Tris-buffered saline with 0.1% Tween 20 (TBST) containing 5% (w/v) bovine serum albumin (Bioshop) or non-fat dry milk (Santa Cruz Biotechnology) for overnight at +4 °C. Membranes were incubated with primary antibodies against SIRT2 (1:500, sc-28298), FOXO3a (1:250, sc-11351), Bim (1:300, sc-11425), Bcl-2 (1:1000, MA1-12,246), Bax (1:500, sc-493), or β-actin (1:125, sc-130657) diluted in TBS for 1–2 h. Membranes were then washed with TBST and incubated for 1 h with their respective horseradish peroxidase-conjugated secondary antibodies (1:5000, sc-2004 or 1:5000, sc-2005). An enhanced chemiluminescence detection reagent (Pierce™ Thermo Sci) was used for visualizing protein bands on X-ray film. The bands were quantified by using the ImageJ software (<http://rsb.info.nih.gov/ij/>) and normalized to β-actin expression levels. The protein expression levels of each group were calculated as fold change to the young control group.

Enzyme-linked Immunosorbent Assay (ELISA)

The quantification of SIRT2 and FOXO3a protein in the cerebral cortex and hippocampus tissues was measured by a sandwich ELISA method. For this purpose commercial ELISA kits (Rat Sirt2 ELISA Kit-ER0299, Wuhan Fine Biological Tech.; Rat Forkhead Box O3A ELISA Kit-MBS9305817, MyBioSource) were used. Tissue samples were prepared by following the manufacturer's protocol. ELISA results were standardized by the total protein content of the samples measured by Bradford's method, and the results were expressed as ng/mg protein for SIRT2 and pg/mg protein for FOXO3a.

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). To examine the effect of age and treatment, a two-way ANOVA was run with the following factors: age (young vs. aged) and treatment (control, melatonin vs. salermide). Post hoc LSD tests were performed to examine the differences between the treatment groups. All respective findings are presented in Tables 1 and 2. Paired-sample *t*-tests were run to compare the cerebral cortex and hippocampus regions. Pearson's *r* was also calculated to investigate the relationships between the variables. Findings of within-subject analysis were presented in the text. The significance level was accepted as $p < 0.05$.

Results

Effect of melatonin on oxidative stress and apoptosis in aging

Compared to young rats, TOS was significantly higher in the cortex and hippocampus of aged rats ($p < 0.001$; Fig. 2a and b), while TAS was significantly lower ($p = 0.004$, $p = 0.001$, respectively; Fig. 2c and d, Table 1). OSI was higher in the cortex and hippocampus of aged rats than young rats ($p < 0.001$; Fig. 2e and f). Melatonin (MLT) administration significantly reduced TOS and OSI in both brain regions compared to control groups in young and aged rats ($p < 0.001$). MLT increased TAS in the cortex and hippocampus of aged rats ($p = 0.02$, $p = 0.004$, respectively), but it had no significant effect on TAS in young rats (Fig. 2, Table 1).

Compared to young rats, Bim in the cortex and Bax in the hippocampus were significantly higher in aged rats ($p = 0.039$, $p < 0.001$, respectively). MLT administration in aged rats significantly reduced Bim in the cortex ($p = 0.022$) and Bax in the hippocampus ($p = 0.002$) compared to the aged control group (Figs. 3a–c, 4a–c, Table 2). The Bcl-2 expression and Bcl-2/Bax ratio were significantly lower in the cortex and hippocampus of aged rats ($p < 0.001$, $p = 0.001$, $p = 0.003$, respectively), whereas MLT increased Bcl-2 and Bcl-2/Bax ratio in the aged group ($p = 0.008$, $p < 0.001$, respectively; Figs. 3d and e, 4d and e).

Salermide (SLM) significantly reduced TOS and OSI in the cortex and hippocampus of young and aged rats compared to control groups ($p < 0.001$), and significantly increased TAS in aged rats ($p = 0.003$, $p < 0.001$, respectively; Fig. 2, Table 1). SLM administration increased Bcl-2 and Bcl-2/Bax ratio in the cortex and hippocampus of aged rats (for Bcl-2 $p = 0.001$, $p = 0.021$; for Bcl-2/Bax $p = 0.008$, $p = 0.001$, respectively) and also increased Bcl-2 in both brain regions of young rats ($p = 0.01$, $p = 0.005$, respectively). SLM reduced increased Bax expression in the hippocampus of aged rats ($p = 0.001$; Fig. 3, 4, Table 2).

Effect of melatonin on SIRT2 and FOXO3a expressions

SIRT2 and FOXO3a expressions were higher in cortex and hippocampus of aged rats compared to young rats (for SIRT2 $p = 0.017$, $p = 0.032$; for FOXO3a $p = 0.007$, $p < 0.001$, respectively), whereas MLT administration decreased SIRT2 and FOXO3a in the hippocampus of the aged rats ($p = 0.028$, $p = 0.036$, respectively), but no significant effect of MLT

Table 1 Changes in parameters related to oxidative stress and in protein levels of SIRT2 and FOXO3a

| | Young | | | Aged | | | F |
|---|--------------|---|---|--|--|---|--|
| | Control | Melatonin | Salermide | Control | Melatonin | Salermide | |
| Cortex | | | | | | | |
| TOS (μmol H ₂ O ₂ Equiv./L) | 13.73 ± 0.14 | 7.13 ± 0.17* <i>t</i> = 25.63, <i>p</i> < 0.001 | 7.67 ± 0.22* <i>t</i> = 23.53, <i>p</i> < 0.001 | 26.37 ± 0.64* <i>t</i> = -19.42, <i>p</i> < 0.001 | 14.68 ± 0.21# <i>t</i> = 20.42, <i>p</i> < 0.001 | 14.3 ± 0.21# <i>t</i> = 21.08, <i>p</i> < 0.001 | <i>F</i> _{age} (1,30) = 1216.38, <i>p</i> < 0.001 <i>F</i> _{treatment} (2,30) = 560.62, <i>p</i> < 0.001 <i>F</i> _{age*treatment} (2,30) = 53.30, <i>p</i> < 0.001 |
| TAS (mmol Trolox Equiv./L) | 2.04 ± 0.07 | 2.06 ± 0.17 <i>t</i> = -0.09, <i>p</i> = 0.928 | 2.16 ± 0.22 <i>t</i> = -0.50, <i>p</i> = 0.623 | 1.37 ± 0.11* <i>t</i> = 2.27, <i>p</i> < 0.001 | 1.82 ± 0.07# <i>t</i> = -2.59, <i>p</i> = 0.020 | 1.97 ± 0.17# <i>t</i> = -3.49, <i>p</i> = 0.003 | <i>F</i> _{age} (1,30) = 9.58, <i>p</i> = 0.004 <i>F</i> _{treatment} (2,30) = 3.13, <i>p</i> = 0.058 <i>F</i> _{age*treatment} (2,30) = 3.35, <i>p</i> = 0.049 |
| OSI | 0.68 ± 0.02 | 0.36 ± 0.02* <i>t</i> = 8.51, <i>p</i> < 0.001 | 0.37 ± 0.03* <i>t</i> = 8.20, <i>p</i> < 0.001 | 2 ± 0.18* <i>t</i> = -7.28, <i>p</i> < 0.001 | 0.82 ± 0.03# <i>t</i> = 7.38, <i>p</i> < 0.001 | 0.7 ± 0.06# <i>t</i> = 7.70, <i>p</i> < 0.001 | <i>F</i> _{age} (1,30) = 110.19, <i>p</i> < 0.001 <i>F</i> _{treatment} (2,30) = 60.13, <i>p</i> < 0.001 <i>F</i> _{age*treatment} (2,30) = 21.55, <i>p</i> < 0.001 |
| SIRT2 (ng/mg protein) | 1 ± 0.1 | 0.98 ± 0.04 <i>t</i> = 0.17, <i>p</i> = 0.865 | 0.91 ± 0.06 <i>t</i> = 0.85, <i>p</i> = 0.409 | 1.27 ± 0.08* <i>t</i> = -2.17, <i>p</i> = 0.050 | 1.2 ± 0.1 <i>t</i> = 0.56, <i>p</i> = 0.582 | 1.14 ± 0.07 <i>t</i> = 1.06, <i>p</i> = 0.308 | <i>F</i> _{age} (1,30) = 14.26, <i>p</i> = 0.001 <i>F</i> _{treatment} (2,30) = 0.93, <i>p</i> = 0.406 <i>F</i> _{age*treatment} (2,30) = 0.07, <i>p</i> = 0.933 |
| FOXO3 (pg/mg protein) | 10.07 ± 1.01 | 10.69 ± 1.45 <i>t</i> = -0.38, <i>p</i> = 0.712 | 5.44 ± 0.93* <i>t</i> = 2.84, <i>p</i> = 0.013 | 13.72 ± 1.54* <i>t</i> = -2.19, <i>p</i> = 0.050 | 14.36 ± 1.79 <i>t</i> = -0.30, <i>p</i> = 0.766 | 9.4 ± 1.06# <i>t</i> = 2.16, <i>p</i> = 0.050 | <i>F</i> _{age} (1,30) = 11.88, <i>p</i> = 0.002 <i>F</i> _{treatment} (2,30) = 8.67, <i>p</i> = 0.001 <i>F</i> _{age*treatment} (2,30) = 3.47, <i>p</i> = 0.044 |
| Hippocampus | | | | | | | |
| TOS (μmol H ₂ O ₂ Equiv./L) | 10.85 ± 0.12 | 5.77 ± 0.09* <i>t</i> = 26.88, <i>p</i> < 0.001 | 6.13 ± 0.18* <i>t</i> = 24.97, <i>p</i> < 0.001 | 20.81 ± 0.49* <i>t</i> = -19.59, <i>p</i> < 0.001 | 11.66 ± 0.18# <i>t</i> = 20.46, <i>p</i> < 0.001 | 11.36 ± 0.15# <i>t</i> = 21.14, <i>p</i> < 0.001 | <i>F</i> _{age} (1,30) = 1256.34, <i>p</i> < 0.001 <i>F</i> _{treatment} (2,30) = 570.70, <i>p</i> < 0.001 <i>F</i> _{age*treatment} (2,30) = 55.51, <i>p</i> < 0.001 |
| TAS (mmol Trolox Equiv./L) | 3.92 ± 0.35 | 4.16 ± 0.13 <i>t</i> = -0.62, <i>p</i> = 0.544 | 4.48 ± 0.28 <i>t</i> = -1.48, <i>p</i> = 0.159 | 2.66 ± 0.18* <i>t</i> = 3.17, <i>p</i> = 0.010 | 3.5 ± 0.17# <i>t</i> = -3.36, <i>p</i> = 0.004 | 4.22 ± 0.19# <i>t</i> = -6.20, <i>p</i> < 0.001 | <i>F</i> _{age} (1,30) = 15.16, <i>p</i> = 0.001 <i>F</i> _{treatment} (2,30) = 10.77, <i>p</i> < 0.001 <i>F</i> _{age*treatment} (2,30) = 5.40, <i>p</i> = 0.009 |
| OSI | 0.29 ± 0.03 | 0.14 ± 0.01* <i>t</i> = 6.04, <i>p</i> < 0.001 | 0.14 ± 0.02* <i>t</i> = 5.97, <i>p</i> < 0.001 | 0.76 ± 0.04* <i>t</i> = -10.23, <i>p</i> < 0.001 | 0.34 ± 0.01# <i>t</i> = 13.16, <i>p</i> < 0.001 | 0.27 ± 0.01# <i>t</i> = 15.14, <i>p</i> < 0.001 | <i>F</i> _{age} (1,30) = 269.43, <i>p</i> < 0.001 <i>F</i> _{treatment} (2,30) = 148.39, <i>p</i> < 0.001 <i>F</i> _{age*treatment} (2,30) = 38.60, <i>p</i> < 0.001 |
| SIRT2 (ng/mg protein) | 1.1 ± 0.08 | 1.4 ± 0.19 <i>t</i> = -1.40, <i>p</i> = 0.184 | 1.16 ± 0.17 <i>t</i> = -0.29, <i>p</i> = 0.773 | 2.05 ± 0.07* <i>t</i> = -8.81, <i>p</i> < 0.001 | 1.54 ± 0.19# <i>t</i> = 2.52, <i>p</i> = 0.024 | 1.11 ± 0.15# <i>t</i> = 4.41, <i>p</i> = 0.001 | <i>F</i> _{age} (1,30) = 8.17, <i>p</i> = 0.008 <i>F</i> _{treatment} (2,30) = 4.75, <i>p</i> = 0.017 <i>F</i> _{age*treatment} (2,30) = 6.57, <i>p</i> = 0.004 |
| FOXO3 (pg/mg protein) | 13.66 ± 1.44 | 11.43 ± 1.1 <i>t</i> = 1.39, <i>p</i> = 0.188 | 13.01 ± 0.85 <i>t</i> = 0.39, <i>p</i> = 0.705 | 15.85 ± 1.96 <i>t</i> = -0.90, <i>p</i> = 0.393 | 14.59 ± 1.36 <i>t</i> = 0.62, <i>p</i> = 0.544 | 11.48 ± 0.75 <i>t</i> = 2.08, <i>p</i> = 0.058 | <i>F</i> _{age} (1,30) = 1.42, <i>p</i> = 0.243 <i>F</i> _{treatment} (2,30) = 1.84, <i>p</i> = 0.176 <i>F</i> _{age*treatment} (2,30) = 1.79, <i>p</i> = 0.184 |

Results are presented as mean ± standard error of the mean (SEM) (**p* < 0.05 compared to Young Control group, # *p* < 0.05 compared to Aged Control group) (n = 6 for each group). All *dfs* for *t*_{LSD} values are equal to 10. Total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI)

was observed in the cortex. MLT also reduced FOXO3a in the hippocampus of young rats (*p* = 0.023; Figs. 3f and g, 4f and g, Table 2). SLM treatment inhibited SIRT2 only

in the hippocampus (*p* = 0.014), but FOXO3a in both brain regions of the aged rats (for cortex *p* = 0.012; for hippocampus *p* = 0.001; Figs. 3f and g, 4f and g).

Table 2 Western blot results normalized by β -actin expression

| | Young | | | Aged | | | F |
|------------------------|-------------|---|--|--|--|--|--|
| | Control | Melatonin | Salermide | Control | Melatonin | Salermide | |
| Cortex | | | | | | | |
| Bim/ β -actin | 0.46 ± 0.13 | 0.49 ± 0.24 <i>t</i> = -0.27, <i>p</i> = 0.791 | 0.62 ± 0.26 <i>t</i> = -1.25, <i>p</i> = 0.229 | 0.78 ± 0.15* <i>t</i> = -3.88, <i>p</i> = 0.003 | 0.53 ± 0.14# <i>t</i> = 2.58, <i>p</i> = 0.022 | 0.67 ± 0.17 <i>t</i> = 1.16, <i>p</i> = 0.265 | $F_{age}(1,30) = 4.68$, <i>p</i> = 0.039 $F_{treatment}(2,30) = 1.51$, <i>p</i> = 0.237 $F_{age*treatment}(2,30) = 3.62$, <i>p</i> = 0.039 |
| Bax/ β -actin | 1.36 ± 0.15 | 1.62 ± 0.67 <i>t</i> = -0.81, <i>p</i> = 0.434 | 1.77 ± 0.62 <i>t</i> = -1.20, <i>p</i> = 0.253 | 1.76 ± 0.53 <i>t</i> = -1.66, <i>p</i> = 0.135 | 1.5 ± 0.62 <i>t</i> = 0.75, <i>p</i> = 0.467 | 1.43 ± 0.5 <i>t</i> = 0.96, <i>p</i> = 0.354 | $F_{age}(1,30) = 0.01$, <i>p</i> = 0.921 $F_{treatment}(2,30) = 0.02$, <i>p</i> = 0.980 $F_{age*treatment}(2,30) = 1.24$, <i>p</i> = 0.304 |
| Bcl-2/ β -actin | 1.46 ± 0.18 | 1.6 ± 0.26 <i>t</i> = -0.98, <i>p</i> = 0.343 | 1.91 ± 0.31* <i>t</i> = -3.00, <i>p</i> = 0.010 | 1.09 ± 0.09* <i>t</i> = 4.59, <i>p</i> = 0.001 | 1.41 ± 0.2# <i>t</i> = -3.05, <i>p</i> = 0.008 | 1.51 ± 0.22# <i>t</i> = -4.01, <i>p</i> = 0.001 | $F_{age}(1,30) = 19.72$, <i>p</i> < 0.001 $F_{treatment}(2,30) = 11.60$, <i>p</i> < 0.001 $F_{age*treatment}(2,30) = 5.61$, <i>p</i> = 0.009 |
| Bcl-2/Bax ratio | 1 ± 0.05 | 0.94 ± 0.09 <i>t</i> = 0.40, <i>p</i> = 0.696 | 0.98 ± 0.13 <i>t</i> = 0.12, <i>p</i> = 0.903 | 0.59 ± 0.06* <i>t</i> = 4.90, <i>p</i> = 0.001 | 0.78 ± 0.08 <i>t</i> = -1.06, <i>p</i> = 0.313 | 1.18 ± 0.18# <i>t</i> = -3.22, <i>p</i> = 0.008 | $F_{age}(1,30) = 1.97$, <i>p</i> = 0.171 $F_{treatment}(2,30) = 3.72$, <i>p</i> = 0.036 $F_{age*treatment}(2,30) = 3.81$, <i>p</i> = 0.034 |
| SIRT2/ β -actin | 0.81 ± 0.12 | 0.9 ± 0.21 <i>t</i> = -0.62, <i>p</i> = 0.549 | 1 ± 0.29 <i>t</i> = -1.37, <i>p</i> = 0.193 | 1.05 ± 0.14* <i>t</i> = -2.92, <i>p</i> = 0.017 | 0.87 ± 0.2 <i>t</i> = 1.90, <i>p</i> = 0.076 | 0.95 ± 0.14 <i>t</i> = 1.01, <i>p</i> = 0.327 | $F_{age}(1,30) = 0.58$, <i>p</i> = 0.452 $F_{treatment}(2,30) = 0.64$, <i>p</i> = 0.534 $F_{age*treatment}(2,30) = 3.58$, <i>p</i> = 0.040 |
| FOXO3a/ β -actin | 0.76 ± 0.04 | 0.63 ± 0.19 <i>t</i> = 1.40, <i>p</i> = 0.184 | 0.61 ± 0.2 <i>t</i> = 1.61, <i>p</i> = 0.130 | 0.99 ± 0.18* <i>t</i> = -2.89, <i>p</i> = 0.016 | 0.9 ± 0.24 <i>t</i> = 0.76, <i>p</i> = 0.457 | 0.65 ± 0.11# <i>t</i> = 2.89, <i>p</i> = 0.012 | $F_{age}(1,30) = 8.37$, <i>p</i> = 0.007 $F_{treatment}(2,30) = 5.27$, <i>p</i> = 0.011 $F_{age*treatment}(2,30) = 3.68$, <i>p</i> = 0.037 |
| Hippocampus | | | | | | | |
| Bim/ β -actin | 0.53 ± 0.12 | 0.54 ± 0.1 <i>t</i> = -0.21, <i>p</i> = 0.839 | 0.58 ± 0.16 <i>t</i> = -0.73, <i>p</i> = 0.480 | 0.58 ± 0.18 <i>t</i> = -0.68, <i>p</i> = 0.514 | 0.56 ± 0.07 <i>t</i> = 0.30, <i>p</i> = 0.770 | 0.41 ± 0.1 <i>t</i> = 2.19, <i>p</i> = 0.053 | $F_{age}(1,30) = 0.45$, <i>p</i> = 0.507 $F_{treatment}(2,30) = 0.68$, <i>p</i> = 0.514 $F_{age*treatment}(2,30) = 2.40$, <i>p</i> = 0.108 |
| Bax/ β -actin | 1.53 ± 0.17 | 1.51 ± 0.43 <i>t</i> = 0.12, <i>p</i> = 0.909 | 1.56 ± 0.31 <i>t</i> = -0.16, <i>p</i> = 0.878 | 2.14 ± 0.1* <i>t</i> = -6.92, <i>p</i> < 0.001 | 1.46 ± 0.33# <i>t</i> = 3.93, <i>p</i> = 0.002 | 1.36 ± 0.33# <i>t</i> = 4.49, <i>p</i> = 0.001 | $F_{age}(1,30) = 1.23$, <i>p</i> = 0.276 $F_{treatment}(2,30) = 5.21$, <i>p</i> = 0.011 $F_{age*treatment}(2,30) = 5.45$, <i>p</i> = 0.010 |
| Bcl-2/ β -actin | 1.36 ± 0.2 | 1.6 ± 0.21 <i>t</i> = -1.59, <i>p</i> = 0.134 | 1.85 ± 0.34* <i>t</i> = -3.28, <i>p</i> = 0.005 | 0.95 ± 0.16* <i>t</i> = 3.76, <i>p</i> = 0.004 | 1.48 ± 0.09# <i>t</i> = -6.08, <i>p</i> < 0.001 | 1.19 ± 0.18# <i>t</i> = -2.64, <i>p</i> = 0.021 | $F_{age}(1,30) = 26.56$, <i>p</i> < 0.001 $F_{treatment}(2,30) = 11.44$, <i>p</i> < 0.001 $F_{age*treatment}(2,30) = 4.67$, <i>p</i> = 0.017 |
| Bcl-2/Bax ratio | 0.89 ± 0.06 | 1.12 ± 0.12 <i>t</i> = -1.53, <i>p</i> = 0.147 | 1.15 ± 0.12 <i>t</i> = -1.74, <i>p</i> = 0.102 | 0.47 ± 0.07* <i>t</i> = 4.83, <i>p</i> = 0.001 | 1.06 ± 0.1# <i>t</i> = -5.57, <i>p</i> < 0.001 | 0.91 ± 0.06# <i>t</i> = -4.43, <i>p</i> = 0.001 | $F_{age}(1,30) = 10.51$, <i>p</i> = 0.003 $F_{treatment}(2,30) = 11.80$, <i>p</i> < 0.001 $F_{age*treatment}(2,30) = 4.46$, <i>p</i> = 0.020 |

Table 2 (continued)

| | Young | | | Aged | | | F |
|------------------------|-----------------|---|---|--|---|---|---|
| | Control | Melatonin | Salermide | Control | Melatonin | Salermide | |
| SIRT2/ β -actin | 0.93 \pm 0.13 | 0.91 \pm 0.14 <i>t</i> = 0.18, <i>p</i> = 0.861 | 1.09 \pm 0.31 <i>t</i> = -1.30, <i>p</i> = 0.212 | 1.37 \pm 0.41* <i>t</i> = -2.49, <i>p</i> = 0.032 | 0.88 \pm 0.29# <i>t</i> = 2.45, <i>p</i> = 0.028 | 0.92 \pm 0.46# <i>t</i> = 2.82, <i>p</i> = 0.014 | $F_{age}(1,30) = 0.13$, <i>p</i> = 0.721 $F_{treatment}(2,30) = 2.80$, <i>p</i> = 0.077 $F_{age*treatment}(2,30) = 5.12$, <i>p</i> = 0.012 |
| FOXO3a/ β -actin | 1 \pm 0.25 | 0.72 \pm 0.13* <i>t</i> = 2.56, <i>p</i> = 0.023 | 0.97 \pm 0.16 <i>t</i> = 0.28, <i>p</i> = 0.787 | 1.41 \pm 0.27* <i>t</i> = -2.69, <i>p</i> = 0.023 | 1.14 \pm 0.16# <i>t</i> = 2.32, <i>p</i> = 0.036 | 0.9 \pm 0.13# <i>t</i> = 4.18, <i>p</i> = 0.001 | $F_{age}(1,30) = 14.24$, <i>p</i> < 0.001 $F_{treatment}(2,30) = 7.57$, <i>p</i> = 0.002 $F_{age*treatment}(2,30) = 5.39$, <i>p</i> = 0.010 |

Results are presented as mean \pm standard error of the mean (SEM) (**p* < 0.05 compared to Young Control group, # *p* < 0.05 compared to Aged Control group) (*n* = 6 for each group). All *d*f's for *t*_{LSD} values are equal to 10

The results obtained by the ELISA method for SIRT2 and FOXO3a were consistent with Western blot results, where we found significant positive correlations between ELISA and Western blot analysis (for SIRT2 *r* = 0.267, *p* = 0.011; for FOXO3a *r* = 0.361, *p* = 0.001). Unlike the western blot results, expression changes in the hippocampal FOXO3a did not differ significantly in ELISA results. Furthermore, SLM treatment in young rats significantly decreased FOXO3a protein levels in the cortex (*p* = 0.013; Fig. 5, Table 1).

Relationship between OSI and expression of SIRT2, FOXO3a and apoptosis-related proteins

OSI was positively correlated with SIRT2, FOXO3a and Bim expressions while negatively correlated with Bcl-2 and Bcl-2/Bax in the cerebral cortex (*r* = 0.450, *p* = 0.002; *r* = 0.401, *p* = 0.006; *r* = 0.405, *p* = 0.006; *r* = -0.598, *p* < 0.001; *r* = -0.389, *p* = 0.014, respectively, Fig. 6). OSI was positively correlated with SIRT2, FOXO3a and Bax expressions while negatively correlated with Bcl-2 and Bcl-2/Bax in the hippocampus (*r* = 0.482, *p* = 0.001; *r* = 0.744, *p* < 0.001; *r* = 0.448, *p* = 0.003; *r* = -0.641, *p* < 0.001; *r* = -0.605, *p* = 0.014, respectively, Fig. 6).

A significant positive correlation between SIRT2 and FOXO3a was observed in both ELISA and Western blot results (*r* = 0.392, *p* < 0.001; *r* = 0.417, *p* < 0.001, respectively). In Western blot results, there was also a positive correlation between FOXO3a and Bim expression in the cortex and hippocampus (*r* = 0.576, *p* < 0.001; *r* = 0.323, *p* = 0.033, respectively, Fig. 6).

Oxidative stress, SIRT2 and FOXO3a expressions in the brain are region-specific

Paired-sample *t*-tests showed that SIRT2 and FOXO3a protein levels were significantly higher in the hippocampus than the cerebral cortex (*t*(35) = 4.09, *p* < 0.001;

t(35) = 2.89, *p* = 0.007, respectively). There was a negative correlation between TOS and TAS in the cortex and hippocampus (*r* = -0.545, *p* < 0.001; *r* = -0.576, *p* < 0.001, respectively). Although TOS and OSI were higher in the cortex at significant levels, TAS was higher in the hippocampus (*t*(35) = 12.34, *p* < 0.001; *t*(35) = 7.09, *p* < 0.001; *t*(35) = 15.79, *p* < 0.001, respectively).

Discussion

Our findings showed that SIRT2 and FOXO3a increased both in the cerebral cortex and hippocampus during the aging process, and this was accompanied by an increase in oxidative stress and apoptosis. Increased SIRT2 expression is considered as one of the specific indicators of premature senescence due to oxidative stress and DNA damage (Anwar et al. 2016). Maxwell et al. suggested that decreased levels of acetylated tubulin correlated with the accumulation of SIRT2 in the aged mouse brain and spinal cord are responsible for initiating neurodegeneration (Maxwell et al. 2011). Braidy et al. demonstrated that SIRT2 mRNA and protein expressions correlated with FOXO3a increased in the occipital lobe, in 24-month-old Wistar rats (Braidly et al. 2015). Our findings also indicated that SIRT2 levels correlated with FOXO3a increased with aging in both brain regions (Figs. 3, 4).

FOXO3a is abundantly expressed in the brain, but limited studies showed its expression in different brain regions in aging. Jackson et al. showed that FOXO3a expression was much higher in the CA1 region which is more sensitive to oxidative and metabolic stressors in aging (Jackson et al. 2009). Exposure to oxidative stress increases FOXO3a expression in the hippocampus (Gómez-Crisóstomo et al. 2014). Liu et al. reported that FOXO3a deacetylation by SIRT2 upregulates Bim expression in PD, which results in increased nigrostriatal pathway damage and apoptosis

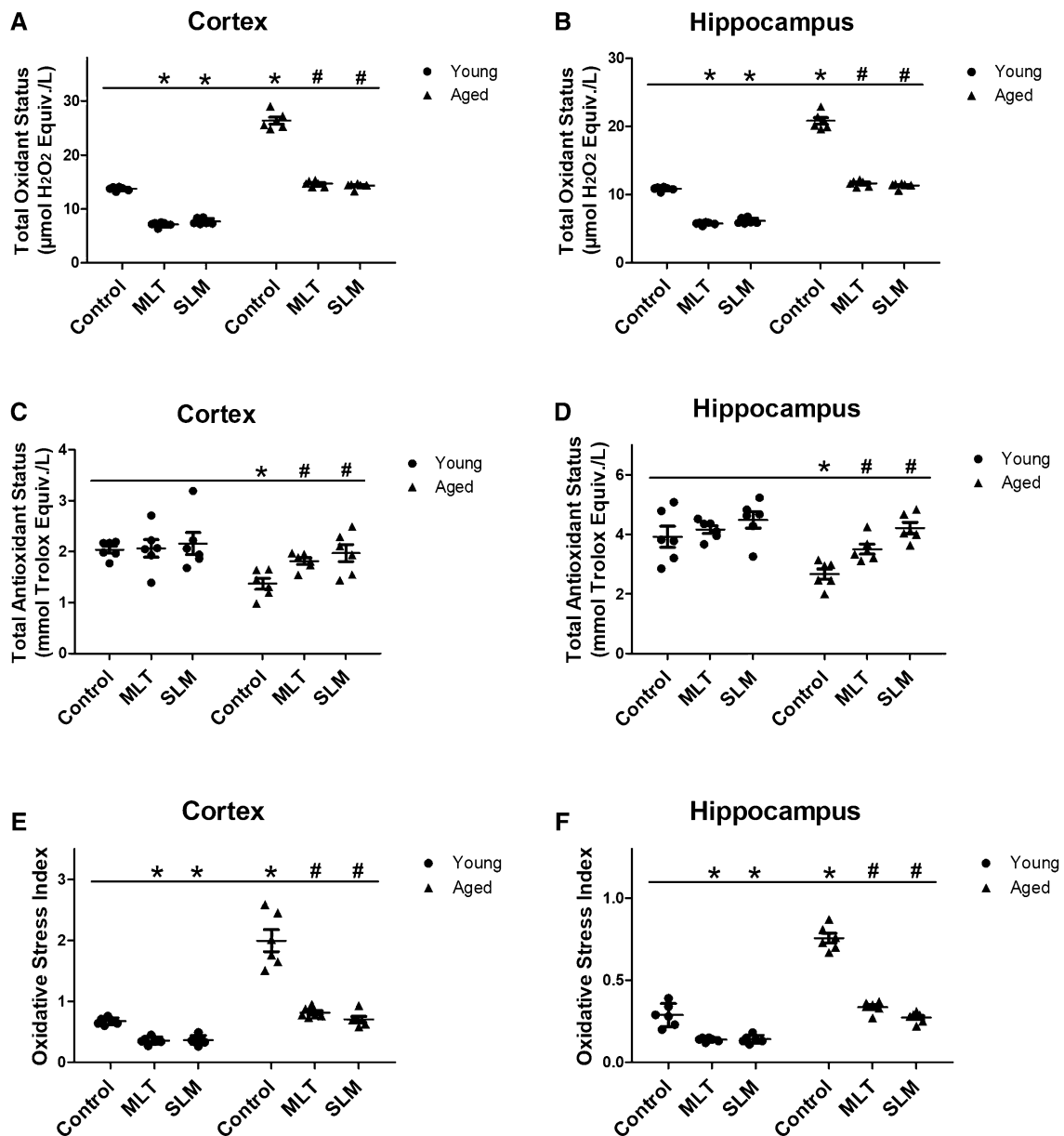


Fig. 2 Total oxidant status (TOS, $\mu\text{mol H}_2\text{O}_2$ Equiv./L) (a, b), total antioxidant status (TAS, mmol Trolox Equiv./L) (c, d), oxidative stress index (OSI) (e, f) in the cortex and hippocampus of young and aged rats treated with melatonin (MLT) or salermide (SLM). Results

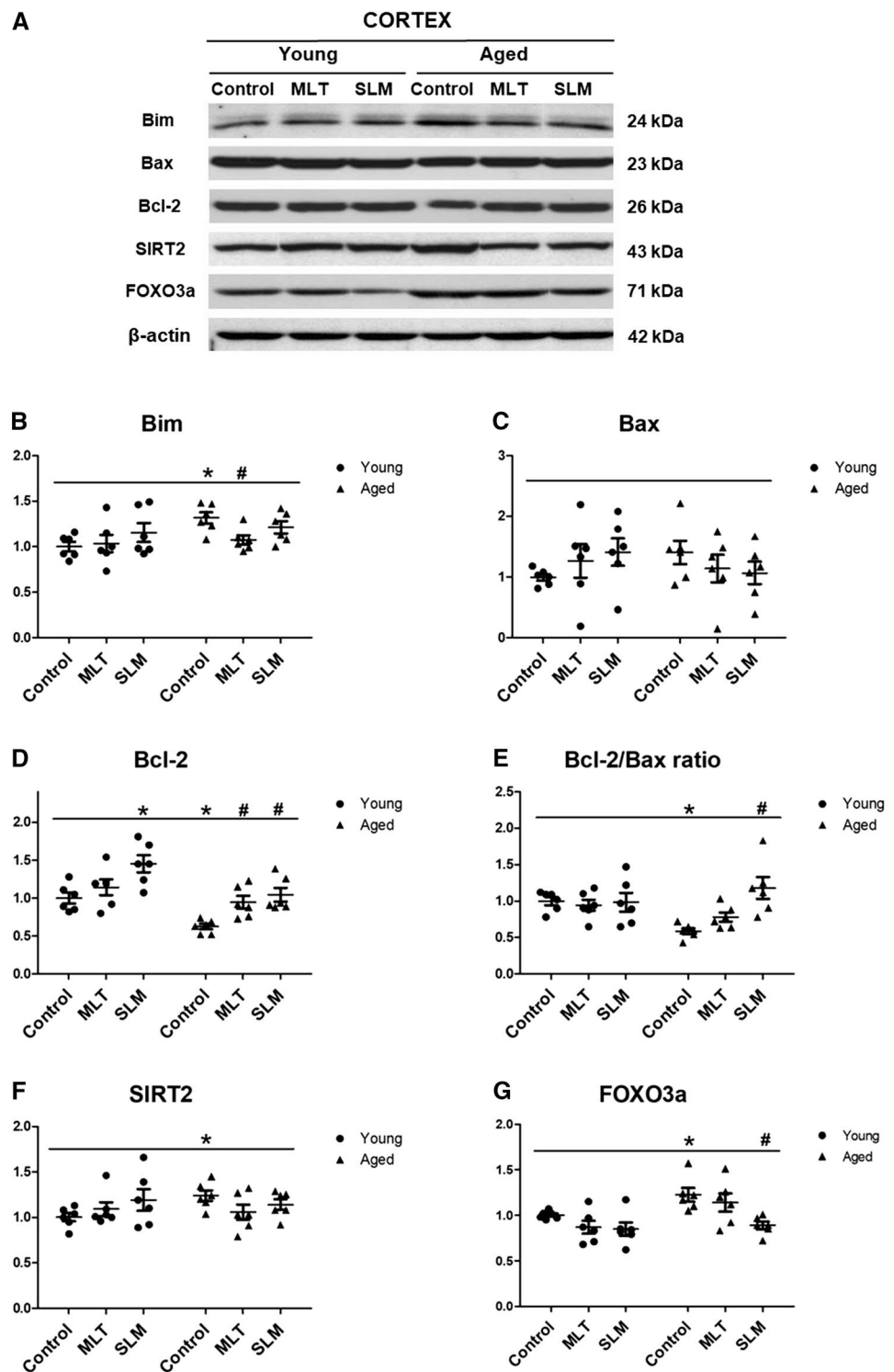
are presented as mean \pm standard error of the mean (SEM). (* $P < 0.05$ compared to Young Control group, # $P < 0.05$ compared to Aged Control group) ($n = 6$ for each group)

(Liu et al. 2014). Qin et al. demonstrated that the IR-PI3K-Akt-FoxO3a pathway involves in amyloid neuropathology. Increased nuclear FOXO3a activity in primary cortical and hippocampal neurons inhibits α -secretase activity and promotes A β production. Therefore, they suggested that FOXO3a inactivation may be effective in reducing amyloid neuropathology and spatial memory disorders (Qin et al. 2008).

The serotonin *N*-acetyltransferase (SNAT) enzyme, which mediates the synthesis of melatonin, and which is shown

to be the largest source of endogenous ROS production in the matrix and intermembranous range of mitochondria. Melatonin synthesis occurs in the mitochondria of various eukaryote cells such as oocytes, pinealocytes, neurons and endothelial cells (Quintela et al. 2018; Tan and Reiter 2019). It was reported in previous studies that there are also melatonin receptors in the neural mitochondria, and mitochondrial MT1 receptors play regulatory roles in the neuroprotective effect of melatonin in ischemic brain damage (Suofu et al. 2017) and Huntingtin-mediated neurotoxicity

Fig. 3 Representative Western blots for Bim, Bax, Bcl-2, SIRT2, FOXO3a and β -actin in the cortex of young and aged rats treated with melatonin (MLT) or salermide (SLM) (a), Bim protein expression levels (b), Bax protein expression levels (c), Bcl-2 protein expression levels (d), Bcl-2/bax ratio (e), SIRT-2 protein expression levels (f) and FOXO3a protein expression levels (g). The protein expression levels were evaluated by Western blotting, and normalized to β -actin expression level and calculated as fold change to the Young Control group. Results are presented as mean \pm standard error of the mean (SEM) (* $P < 0.05$ compared to Young Control group, # $P < 0.05$ compared to Aged Control group) ($n = 6$ for each group)

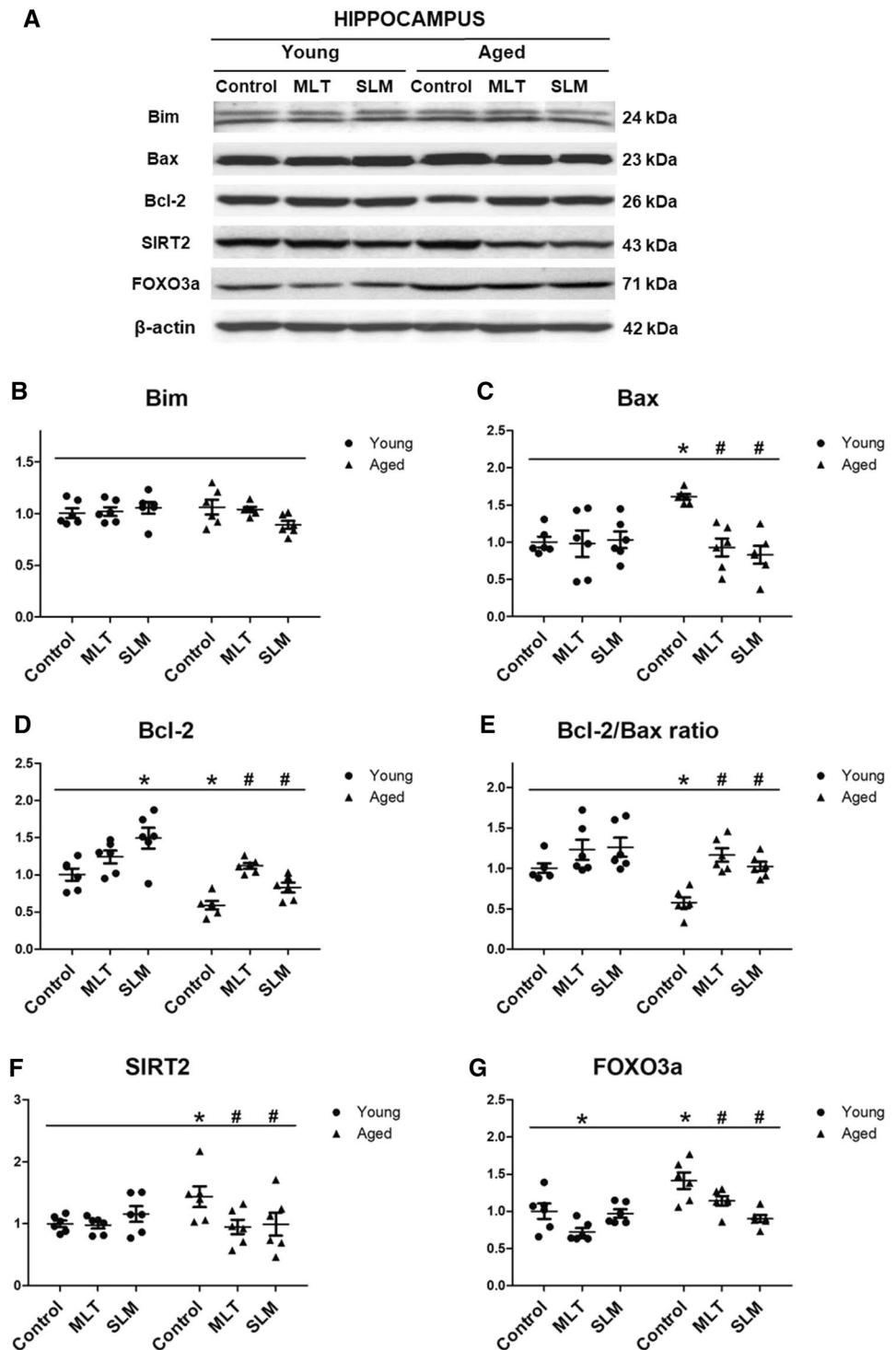


(Xin Wang et al. 2011). Decreased melatonin levels in aging are considered to be one of the most important causes of neurodegeneration and neural loss (Reiter et al. 2018). Melatonin administration downregulates PTEN and FOXO3a in $A\beta_{25-35}$ -induced toxicity by rescuing miR-132 expression (Zhao et al. 2018). Melatonin increases the binding affinity

of FOXO3a for the p27^{Kip1} promoter by suppressing phosphorylation and cytoplasmic translocation of FOXO3a (Jang et al. 2017).

Oxidative stress leads to an increase in total oxidant status in the brain tissue and plasma and a decrease in total antioxidant status (Atmaca et al. 2014). Lipid peroxidation,

Fig. 4 Representative Western blots for Bim, Bax, Bcl-2, SIRT2, FOXO3a and β -actin in the hippocampus of young and aged rats treated with melatonin (MLT) or salermide (SLM) (a), Bim protein expression levels (b), Bax protein expression levels (c), Bcl-2 protein expression levels (d), Bcl-2/bax ratio (e), SIRT-2 protein expression levels (f) and FOXO3a protein expression levels (g). The protein expression levels were evaluated by Western blotting, and normalized to β -actin expression level and calculated as fold change to the Young Control group. Results are presented as mean \pm standard error of the mean (SEM) (* $P < 0.05$ compared to Young Control group, # $P < 0.05$ compared to Aged Control group) ($n = 6$ for each group)



protein oxidation and nitration increase in the cerebral cortex, hippocampus, cerebellum and limbic areas in aging, whereas antioxidant systems decrease (Akbulut et al. 2008; Keskin-Aktan et al. 2018; Kireev et al. 2013; Venkateshappa et al. 2012). Melatonin has been shown to decrease oxidative stress and apoptosis in different brain regions (Akbulut et al. 2008; Kireev et al. 2013; Paredes et al. 2015). Our findings

showed that TOS, OSI, pro-apoptotic protein expressions (Bim in the cortex, Bax in the hippocampus) increased in the cortex and hippocampus of aged rats, while TAS, Bcl-2 expression and Bcl-2/Bax ratio decreased. Increased oxidative damage and apoptotic activity in the cerebral cortex and hippocampus have been associated with Bcl-2 down-regulation and Bax up-regulation (Xu et al. 2007). Weinreb

Fig. 5 SIRT2 protein levels (ng/mg protein) in the cortex (a) and hippocampus (b) of young and aged rats treated with melatonin (MLT) or salermide (SLM). FOXO3a protein levels (pg/mg protein) in the cortex (c) and hippocampus (d) of young and aged rats treated with MLT or SLM. Results are presented as mean \pm standard error of the mean (SEM) (* $P < 0.05$ compared to the Young Control group, # $P < 0.05$ compared to the Aged Control group) ($n = 6$ for each group)

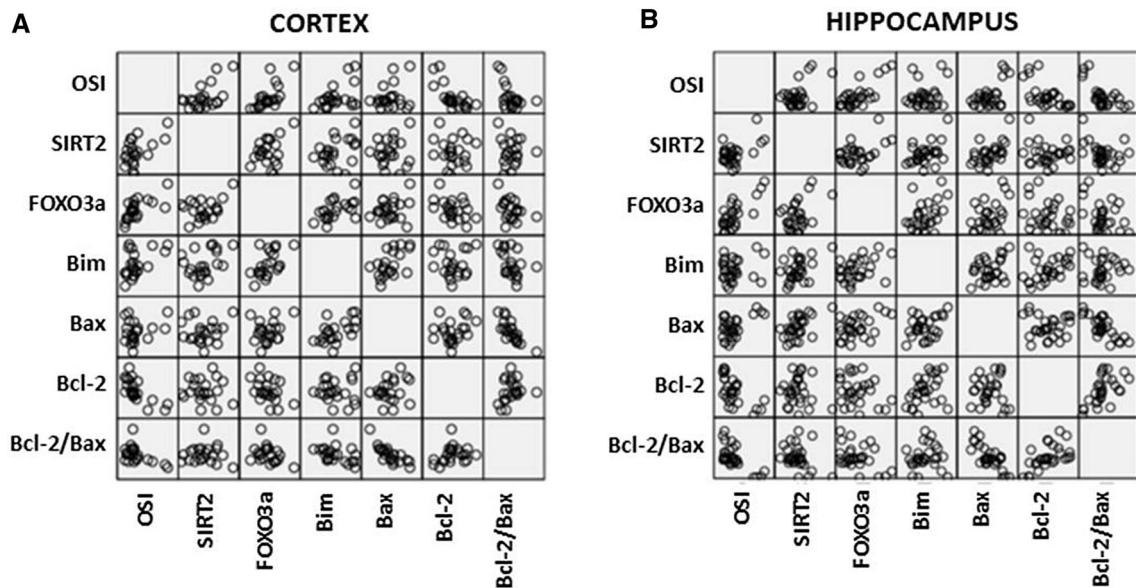
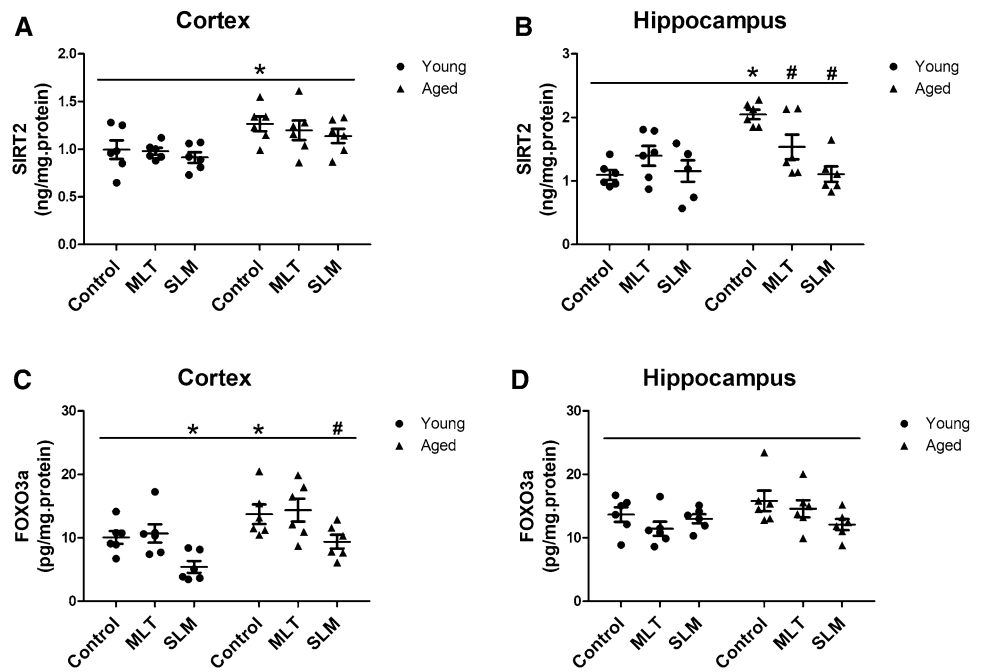


Fig. 6 Relationship between OSI and expression of SIRT2, FOXO3a and apoptosis-related proteins. Pearson’s correlation test results showed that OSI was positively correlated with SIRT2, FOXO3a and Bim expressions while negatively correlated with Bcl-2 and Bcl-2/Bax in the cerebral cortex. OSI was positively correlated with SIRT2,

FOXO3a and Bax expressions while negatively correlated with Bcl-2 and Bcl-2/Bax in the hippocampus. There was also a positive correlation between FOXO3a and Bim expression in the cortex and hippocampus ($n = 36, P < 0.05$)

et al. reported that Bcl-2, BDNF, synapsins 1 and Trk-B receptor mRNA expressions decreased in the hippocampus with aging, but they observed no significant changes in Bax expression (Weinreb et al. 2015). Kireev et al. showed that pro-apoptotic proteins such as Bax, Bak, AIF increased with aging in the dentate gyrus, whereas anti-apoptotic proteins

such as XIAP, NIAP, Mcl-1 decreased (Kireev et al. 2013). Melatonin enhances the antioxidants (GSH, SOD, catalase, etc.) and detoxification gene expressions by receptor-dependent and/or independent pathways in aging and various environmental stress conditions. Melatonin can also increase the redox-sensitive Bcl-2 and Bcl-2/Bax ratio by

its anti-apoptotic and antioxidant properties (Caballero et al. 2009). Melatonin induces apoptosis by reducing the Bcl-2/Bax ratio in cancer cells (Leja-Szpak et al. 2010) while preventing neural loss by Bcl-2 up-regulation and Bax down-regulation in homocysteine-induced hippocampal cell death (Baydas et al. 2005).

Our study found that melatonin decreased TOS, OSI and expressions of Bim (in the cortex) and Bax (in the hippocampus) while increasing TAS and Bcl-2 in both brain regions of aged rats. Salermide revealed melatonin-like effects in our study, decreasing TOS and OSI levels in both brain regions in aged rats while increasing TAS and Bcl-2 levels. Furthermore, like melatonin, salermide also reduced Bax in the hippocampus. The effects of salermide are revealed through SIRT2 inhibition (Alcaín and Villalba, 2009; Lara et al. 2009; Peck et al. 2010). Similar effects of salermide and melatonin on TAS, TOS and OSI suggest that SIRT2 inhibition plays a role in reducing oxidative stress in aging. SIRT2 inhibition reduces oxidative stress, inflammation, cytokines, MAPK and JNK (c-Jun N-terminal kinase) phosphorylation (Anwar et al. 2016; Sarikhani et al. 2018; She et al. 2018). Melatonin and salermide were also found to be effective in reducing TOS and OSI in young rats, but the enhancing effect on TAS was not significant, it may be necessary to increase the sample size.

Pro-apoptotic Bim, which is associated with microtubules, is mainly controlled by FOXO3a, and an increase in FOXO3a-mediated Bim expression leads to apoptosis (Cabaj-Pescador et al. 2013; Wang et al. 2007). Similarly, we observed an increase in Bim correlated with FOXO3a in the cerebral cortex of aged rats. JNK-mediated phosphorylation of Bim leads to neural apoptosis (Becker et al. 2004), and A β peptide-induced Bim results in loss of hippocampal and cortical neurons in AD (Biswas et al. 2007). Shimmyo et al. showed that Bid increased in the early stages of HD, while Bim in the later stages, and on this basis, they suggested that Bim is an important indicator of the progression and severity of neuronal dysfunction (Shimmyo et al. 2008). To our knowledge, no study showed aging-related changes in Bim expression in the brain, except for studies showing a Bim increase in the heart (Sin et al. 2014) and skeletal muscle in aging (Sin et al. 2015).

Oxidative stress induced by H₂O₂ increases FOXO3a in quiescent cells (Kops et al. 2002). SIRT2 can increase cell death by deacetylating the FOXO family. Wang et al. showed that increased SIRT2 expression in response to H₂O₂-induced oxidative stress upregulates expression of FOXO3a target genes such as MnSOD, p27^{Kip1} and Bim (F. Wang et al. 2007). Overexpression of SIRT2 induces cell death, while SIRT2 inhibition increases tolerance to oxidative stress (Lynn et al. 2008). Oxidative stress and apoptosis can be suppressed by AGK-2 mediated SIRT2 inhibition (Nie et al. 2014). Therefore, SIRT2 is regarded

as a mediator in oxidative stress-induced cell death. Li et al. also showed that SIRT2, Bax and Bim were overexpressed in MPP-induced dopaminergic neuron loss and miR-7 treatment, directly targeted to SIRT2 and Bax, reduced neural losses (Li et al. 2016). SIRT2 inhibitor AGK-2 treatment in thioacetamide-induced liver toxicity ameliorates the toxicity and suppresses apoptosis by decreasing Bax and increasing Bcl-2 (Jiao et al. 2019). On the other hands, overexpression of SIRT2 in glioma cells upregulates caspase and Bax protein and downregulates Bcl-2 (Y. Li et al. 2013).

We found that melatonin and salermide inhibited increased FOXO3a expression as well as SIRT2 in the hippocampus of aged rats. ELISA and western blot results were positively correlated for SIRT2 and FOXO3a levels in both brain regions. Although significant differences between groups were similar in ELISA and western blot results, significant differences in western blot results were not observed in ELISA results only for hippocampal FOXO3a. This may be related to methodological differences and/or sample size (Figs. 3, 4, 5). Although its mechanism has not been elucidated yet, the regulatory effects of melatonin in physiological and pathological conditions include sirtuin and FOXO proteins. In our previous studies, we showed that melatonin is effective in reducing reactive oxygen species, regulating the expression of apoptosis-related proteins such as Bcl-2, Bax and caspase 3 (Akbulut et al. 2008, 2012; Keskin-Aktan et al. 2018), as well as providing inhibition of SIRT2 expression and oxidative stress in the adult rat hippocampus (Keskin-Aktan et al. 2018). Zhao et al. showed that melatonin inhibits neurotoxicity by suppressing FOXO3a via miR-132/PTEN/AKT/FOXO3a pathway (Zhao et al. 2018). On the other hands, Jenwitheesuk et al. reported that melatonin administration increased p27 and FOXO3a mRNA expressions in the hippocampus in 19-month-old mice (Jenwitheesuk et al. 2018).

The hippocampus and neocortex are the most sensitive brain regions to aging and neurodegeneration. Our results indicate that the responses to aging and exogenous treatments are not uniform in the brain. Morphological, metabolic and genomic features of the brain are region-specific, and it is known that the vulnerability of each region, even sub-regions, to aging, oxidative stress and inflammation are different (Clarke et al. 2018; Fjell et al. 2014; Xinkun Wang and Michaelis, 2010). Astrocytes are the most abundant glial cells in the CNS, and astrocyte dysfunction is associated with age-related neurodegenerative diseases such as AD, PD and dementia (Clarke et al. 2018). The astrocyte count, astrocyte/neuron ratio and astrocyte/microglia ratio are different in the cerebral cortex and hippocampus. Neuron density is less in the hippocampus compared to the cortex, while the astrocyte/neuron ratio is higher (Keller et al. 2018). The hippocampus is more vulnerable to oxidative stress compared to the

cortex (Candelario-Jalil et al. 2001). Although there are widespread expressions of melatonin G-protein coupled receptors (MT1, MT2) in the central nervous system, the distribution of these receptors is region-specific. MT1 has higher immunoreactivity in the cerebral cortex (prefrontal, retrosplenial, and occipital), and MT2 especially has higher immunoreactivity in the hippocampus (CA3 and dentate gyrus) (Ng et al. 2017). It was shown that MT2 immunoreactivity increases in astrocytes rather than in the microglia in neuronal damage of CA1, which is highly sensitive to ischemic damage (Lee et al. 2010). Our findings indicated that SIRT2 and FOXO3a protein levels are significantly higher in the hippocampus than in the cerebral cortex. The reason why the SIRT2 and FOXO3a levels in the cortex of aged rats did not decrease significantly with melatonin may be related to the genomic and metabolic differences of the cortex and hippocampus. Due to the regional differences in the brain, the dosage of melatonin and salermide treatment used in the present study was sufficient for SIRT2 inhibition in the hippocampus of aged rats, but it might not be sufficient for a significant reduction in the cortex.

In the present study, OSI was positively correlated with SIRT2, FOXO3a, Bim (in the cortex) and Bax (in the hippocampus) while negatively correlated with Bcl-2 and Bcl-2/Bax in both brain regions. The exogenous melatonin significantly decreased TOS, OSI, SIRT2, FOXO3a, Bim (in the cortex) and Bax (in the hippocampus) (Figs. 2, 3, 4). Accordingly, salermide-mediated SIRT2 inhibition was also accompanied by a decrease in TOS, OSI and Bax, and an increase in TAS, Bcl-2 and Bcl-2/Bax in the hippocampus (Figs. 2, 3, 4).

In conclusion, our study suggests that SIRT2 and FOXO3a act as key regulators of oxidative stress and apoptosis. Inhibition of SIRT2 and FOXO3a seems to be involved in the protective effects of melatonin in the aged rat brain, especially in the hippocampus. Thus, SIRT2 and FOXO3a may be potential therapeutic targets in aging and neurodegeneration. However, further studies are needed to investigate the effect of melatonin on aging-related signaling pathways.

Author's contributions AKA, KGA and HA designed the research study; AKA, KGA and SA performed the experiments; AKA and HA analyzed the data; AKA and KGA wrote the paper; AKA, KGA and HA participated in the conception of the study and revision of the manuscript. All authors approve of the final version to be published.

Funding The Scientific and Technological Research Council of Turkey (TÜBİTAK project no. 216S258) and Gazi University Scientific Research Project Foundation (GÜBAP project no. 01/2016–05) provided the financial support for this study.

Data availability All data presented during this research are included in this article.

Declarations

Conflict of interest The authors report no conflict of interest.

Ethics approval Animals were treated according to the guidelines of the European Convention ETS 123, and all the methods used in the present study were approved by the Animal Experiments Ethics Committee of Gazi University (#G.U.ET-16.008).

References

- Akbulut KG, Gonul B, Akbulut H (2008) Exogenous melatonin decreases age-induced lipid peroxidation in the brain. *Brain Res* 1238:31–35. <https://doi.org/10.1016/j.brainres.2008.08.014>
- Akbulut KG, Akbulut H, Akgun N, Gonul B (2012) Melatonin decreases apoptosis in gastric mucosa during aging. *Aging Clin Exp Res* 24:15–20. <https://doi.org/10.3275/7568>
- Alcaín FJ, Villalba JM (2009) Sirtuin Inhibitors. *Expert Opin Therapeutic Patents* 19(3):283–294. <https://doi.org/10.1517/13543770902755111>
- Anwar T, Khosla S, Ramakrishna G (2016) Increased expression of SIRT2 is a novel marker of cellular senescence and is dependent on wild type p53 status. *Cell Cycle* 15(14):1883–1897. <https://doi.org/10.1080/15384101.2016.1189041>
- Atmaca N, Tarik H, Kanici A, Antepioglu T (2014) Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity in rats. *Food Chem Toxicol J* 70:191–197. <https://doi.org/10.1016/j.fct.2014.05.011>
- Baydas G, Reiter RJ, Akbulut M, Tuzcu M, Tamer S (2005) Melatonin inhibits neural apoptosis induced by homocysteine in hippocampus of rats via inhibition of cytochrome c translocation and caspase-3 activation and by regulating pro- and anti-apoptotic protein levels. *Neuroscience* 135:879–886. <https://doi.org/10.1016/j.neuroscience.2005.05.048>
- Becker EBE, Howell J, Kodama Y, Barker PA, Bonni A (2004) Characterization of the c-Jun N-Terminal Kinase-BimEL signaling pathway in neuronal apoptosis. *J Neurosci* 24(40):8762–8770. <https://doi.org/10.1523/JNEUROSCI.2953-04.2004>
- Biella G, Fusco F, Nardo E, Bernocchi O, Colombo A, Lichtenthaler SF, Forloni G, Albani D (2016) Sirtuin 2 inhibition improves cognitive performance and acts on Amyloid- β protein precursor processing in two Alzheimer's disease mouse models. *J Alzheimer's Dis* 53(3):1193–1207. <https://doi.org/10.3233/JAD-151135>
- Biswas SC, Shi Y, Vonsattel J-PG, Leung CL, Troy CM, Greene LA (2007) Bim is elevated in Alzheimer's disease neurons and is required for -amyloid-induced neuronal apoptosis. *J Neurosci* 27(4):893–900. <https://doi.org/10.1523/JNEUROSCI.3524-06.2007>
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72(1–2):248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Braidy N, Poljak A, Grant R, Jayasena T, Mansour H, Chan-Ling T, Smythe G, Sachdev P, Guillemin GJ (2015) Differential expression of sirtuins in the aging rat brain. *Front Cellular Neurosci* 9(167):1–16. <https://doi.org/10.3389/fncel.2015.00167>
- Caballero B, Vega-Naredo I, Sierra V, Huidobro-Fernandez C, Soria-Valles C, Gonzalo-Calvo D, Tolivia D, Pallas M, Camins A, Rodriguez-Colunga MJ, Coto-Montes A (2009) Melatonin alters cell death processes in response to age-related oxidative stress in the brain of senescence-accelerated mice. *J Pineal Res* 46:106–114. <https://doi.org/10.1111/j.1600-079X.2008.00637.x>

- Candelario-Jalil E, Mhadu NH, Al-Dalain SM, Martínez G, León OS (2001) Time course of oxidative damage in different brain regions following transient cerebral ischemia in gerbils. *Neurosci Res* 41(3):233–241. [https://doi.org/10.1016/S0168-0102\(01\)00282-6](https://doi.org/10.1016/S0168-0102(01)00282-6)
- Carbajo-Pescador S, Steinmetz C, Kashyap A, Lorenz S, Mauriz JL, Heise M, Galle PR, González-Gallego J, Strand S (2013) Melatonin induces transcriptional regulation of Bim by FoxO3a in HepG2 cells. *Br J Cancer* 108(2):442–449. <https://doi.org/10.1038/bjc.2012.563>
- Chen X, Wales P, Quinti L, Zuo F, Moniot S, Herisson F (2015) The Sirtuin-2 inhibitor AK7 is Neuroprotective in models of Parkinson's disease but not amyotrophic lateral sclerosis and cerebral ischemia. *PLoS ONE* 10(1):e0116919.
- Clarke LE, Liddelow SA, Chakraborty C, Münch AE, Heiman M, Barres BA (2018) Normal aging induces A1-like astrocyte reactivity. *Proc Natl Acad Sci* 115(8):E1896–E1905. <https://doi.org/10.1073/pnas.1800165115>
- Fjell AM, McEvoy L, Holland D, Dale AM, Walhovd KB (2014) What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. *Progress Neurobiol* 117:20–40. <https://doi.org/10.1016/j.pneurobio.2014.02.004>
- Gómez-Crisóstomo NP, Rodríguez Martínez E, Rivas-Arancibia S (2014) Oxidative stress activates the transcription factors FoxO1a and FoxO3a in the hippocampus of rats exposed to low doses of ozone. *Oxidative Med Cellular Longevity* 2014:1–8. <https://doi.org/10.1155/2014/805764>
- Greer EL, Brunet A (2005) FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene* 24(50):7410–7425. <https://doi.org/10.1038/sj.onc.1209086>
- Huang PS, Son JH, Abbott LC, Winzer-Serhan UH (2011) Regulated expression of neuronal SIRT1 and related genes by aging and neuronal β 2-containing nicotinic cholinergic receptors. *Neuroscience* 196:189–202. <https://doi.org/10.1016/j.neuroscience.2011.09.007>
- Jackson TC, Rani A, Kumar A, Foster TC (2009) Regional hippocampal differences in AKT survival signaling across the lifespan: Implications for CA1 vulnerability with aging. *Cell Death Differentiation* 16(3):439–448. <https://doi.org/10.1038/cdd.2008.171>
- Jang H, Na Y, Hong K, Lee S, Moon S, Cho M, Park M, Lee OH, Chang EM, Lee DR, Ko JJ, Lee WS, Choi Y (2017) Synergistic effect of melatonin and ghrelin in preventing cisplatin-induced ovarian damage via regulation of FOXO3a phosphorylation and binding to the p27Kip1 promoter in primordial follicles. *J Pineal Res* 63(3):1–17. <https://doi.org/10.1111/jpi.12432>
- Jenwitheesuk A, Park S, Wongchitrat P, Tocharus J, Mukda S, Shimokawa I, Govitrapong P (2018) Comparing the effects of melatonin with caloric restriction in the hippocampus of aging mice: involvement of Sirtuin1 and the FOXOs pathway. *Neurochem Res* 43(1):144–152. <https://doi.org/10.1007/s11064-017-2369-7>
- Jiao FZ, Wang Y, Zhang WB, Zhang HY, Chen Q, Shi CX, Wang LW, Gong ZJ (2019) Protective role of AGK2 on thioacetamide-induced acute liver failure in mice. *Life Sci* 230(April):68–75. <https://doi.org/10.1016/j.lfs.2019.05.061>
- Keller D, Erö C, Markram H (2018) Cell densities in the mouse brain: A systematic review. *Front Neuroanatomy* 12(October). <https://doi.org/10.3389/fnana.2018.00083>
- Keskin-Aktan A, Akbulut KGKG, Yazici-Mutlu Ç, Sonugur G, Ocal M, Akbulut H (2018) The effects of melatonin and curcumin on the expression of SIRT2, Bcl-2 and Bax in the hippocampus of adult rats. *Brain Res Bull* 137:306–310. <https://doi.org/10.1016/j.brainresbull.2018.01.006>
- Kireev RA, Vara E, Tresguerres JAF (2013) Growth hormone and melatonin prevent age-related alteration in apoptosis processes in the dentate gyrus of male rats. *Biogerontology* 14(4):431–442. <https://doi.org/10.1007/s10522-013-9443-6>
- Kops GJPL, Dansen TB, Polderman PE, Saarloos I, Wirtz KWA, Coffey PJ, Huang TT, Bos JL, Medema RH, Burgering BMT (2002) Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419(6904):316–321. <https://doi.org/10.1038/nature01036>
- Lara E, Mai A, Calvanese V, Altucci L, Lopez-Nieva P, Martinez-Chantar ML, Varela-Rey M, Rotili D, Nebbioso A, Ropero S, Montoya G, Oyarzabal J, Velasco S, Serrano M, Witt M, Villar-Garea A, Imhof A, Mato JM, Esteller M, Fraga MF (2009) Salmeterol, a sirtuin inhibitor with a strong cancer-specific proapoptotic effect. *Oncogene* 28(6):781–791. <https://doi.org/10.1038/onc.2009.1>
- Lee CH, Yoo KY, Choi JH, Park OK, Hwang IK, Kwon YG, Kim YM, Won MH (2010) Melatonin's protective action against ischemic neuronal damage is associated with up-regulation of the MT2 melatonin receptor. *J Neurosci Res* 88(12):2630–2640. <https://doi.org/10.1002/jnr.22430>
- Leja-Szpak A, Jaworek J, Pierzchalski P, Reiter RJ (2010) Melatonin induces pro-apoptotic signaling pathway in human pancreatic carcinoma cells (PANC-1). *J Pineal Res* 49(3):248–255. <https://doi.org/10.1111/j.1600-079X.2010.00789.x>
- Li Y, Dai D, Lu Q, Fei M, Li M, Wu X (2013) Biochemical and Biophysical Research Communications Sirt2 suppresses glioma cell growth through targeting NF- κ B – miR-21 axis. *Biochem Biophys Res Commun* 441:661–667. <https://doi.org/10.1016/j.bbrc.2013.10.077>
- Li S, Lv X, Zhai K, Xu R, Zhang Y, Zhao S, Qin X, Yin L (2016) MicroRNA-7 inhibits neuronal apoptosis in a cellular Parkinson's disease model by targeting Bax and Sirt2. *Am J Transl Res* 8(2):993–1004
- Liu L, Arun A, Ellis L, Peritore C, Donmez G (2014) SIRT2 enhances 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigrostriatal damage via apoptotic pathway. *Front Aging Neurosci* 6(JUL):1–7. <https://doi.org/10.3389/fnagi.2014.00184>
- Luthi-Carter R, Taylor DM, Pallos J, Lambert E, Amore A, Parker A (2010) SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. *PNAS* 107(17):7927–7932. <https://doi.org/10.1073/pnas.1002924107>
- Lynn EG, McLeod CJ, Gordon JP, Bao J, Sack MN (2008) SIRT2 is a negative regulator of anoxia—reoxygenation tolerance via regulation of 14-3-3 f and BAD in H9c2 cells. *FEBS Lett* 582:2857–2862. <https://doi.org/10.1016/j.febslet.2008.07.016>
- Maxwell MM, Tomkinson EM, Nobles J, Wizeman JW, Amore AM, Quinti L, Chopra V, Hersch SM, Kazantsev AG (2011) The Sirtuin 2 microtubule deacetylase is an abundant neuronal protein that accumulates in the aging CNS. *Hum Mol Genet* 20(20):1–11. <https://doi.org/10.1093/hmg/ddr326>
- Michan S, Sinclair D (2007) Sirtuins in mammals: insights into their biological function. *Biochem J* 404(1):1–13. <https://doi.org/10.1042/BJ20070140>
- Ng KY, Leong MK, Liang H, Paxinos G (2017) Melatonin receptors: distribution in mammalian brain and their respective putative functions. *Brain Structure Function* 222(7):2921–2939. <https://doi.org/10.1007/s00429-017-1439-6>
- Nie H, Hong Y, Lu X, Zhang J, Chen H, Li Y, Ma Y, Ying W (2014) SIRT2 mediates oxidative stress-induced apoptosis of differentiated PC12 cells. *NeuroReport* 25:838–842. <https://doi.org/10.1097/WNR.0000000000000192>
- Öztürk G, Akbulut KG, Güney Ş, Acuna-Castroviejo D (2012) Age-related changes in the rat brain mitochondrial antioxidative enzyme ratios: modulation by melatonin. *Exp Gerontol* 47:706–711
- Paredes SD, Rancan L, Kireev R, González A, Louzao P, González P, Rodríguez-Bobada C, García C, Vara E, Tresguerres JAFF (2015) Melatonin counteracts at a transcriptional level the inflammatory and apoptotic response secondary to ischemic brain injury induced

- by middle cerebral artery blockade in aging rats. *BioRes Open Access* 4(1):407–416. <https://doi.org/10.1089/biores.2015.0032>
- Park JH, Lee CH, Yoo KY, Choi JH, Hwang IK, Lee JY, Kang IJ, Won MH (2011) FoxO3a immunoreactivity is markedly decreased in the dentate gyrus, not the hippocampus proper, of the aged gerbil. *Exp Gerontol* 46(10):836–840. <https://doi.org/10.1016/j.exger.2011.06.001>
- Peck B, Chen C-Y, Ho K-K, Di Fruscia P, Myatt SS, Coombes RC, Fuchter MJ, Hsiao C-D, Lam EW-F (2010) SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. *Mol Cancer Ther* 9(4):844–855. <https://doi.org/10.1158/1535-7163.MCT-09-0971>
- Pino E, Amamoto R, Zheng L, Cacquevel M, Sarria JC, Knott GW, Schneider BL (2014) FOXO3 determines the accumulation of alpha-synuclein and controls the fate of dopaminergic neurons in the substantia nigra. *Hum Mol Genet* 23(6):1435–1452. <https://doi.org/10.1093/hmg/ddt530>
- Pollack M, Phaneuf S, Dirks A (2002) The role of apoptosis in the normal aging brain, skeletal muscle, and heart. *Ann NY Acad Sci* 959:93–107
- Qin W, Zhao W, Ho L, Wang J, Walsh K, Gandy S, Pasinetti GM (2008) Regulation of forkhead transcription factor FoxO3a contributes to calorie restriction-induced prevention of Alzheimer's disease-type amyloid neuropathology and spatial memory deterioration. *Ann N Y Acad Sci* 1147:335–347. <https://doi.org/10.1196/annals.1427.024>
- Quintela T, Gonçalves I, Silva M, Duarte AC, Guedes P, Andrade K, Freitas F, Talhada D, Albuquerque T, Tavares S, Passarinha LA, Cipolla-Neto J, Santos CRA (2018) Choroid plexus is an additional source of melatonin in the brain. *J Pineal Res* 65(4):1–9. <https://doi.org/10.1111/jpi.12528>
- Reiter RJ, Tan DX, Rosales-Corral S, Galano A, Zhou XJ, Xu B (2018) Mitochondria: central organelles for melatonins antioxidant and anti-aging actions. *Molecules* 23(2):1–25. <https://doi.org/10.3390/molecules23020509>
- Sarikhani M, Mishra S, Desingu PA, Kotyada C, Wolfgeher D, Gupta MP, Singh M, Sundaresan NR (2018) SIRT2 regulates oxidative stress-induced cell death through deacetylation of c-Jun NH2-terminal kinase. *Cell Death Differentiation* 25(9):1638–1656. <https://doi.org/10.1038/s41418-018-0069-8>
- She DT, Wong LJ, Baik S, Arumugam TV (2018) SIRT2 Inhibition Confers Neuroprotection by Downregulation of FOXO3a and MAPK Signaling Pathways in Ischemic Stroke. *Molecular Neurobiol*, 1–16.
- Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H (2008) Multifunction of Myricetin on Ab: Neuroprotection via a conformational change of Ab and reduction of Ab via the Interference of Secretases. *J Neurosci Res* 86:368–377. <https://doi.org/10.1002/jnr>
- Sin TK, Yu AP, Yung BY, Yip SP, Chan LW, Wong CS, Ying M, Rudd JA, Siu PM (2014) Modulating effect of SIRT1 activation induced by resveratrol on Foxo1-associated apoptotic signalling in senescent heart. *J Physiol* 592(12):2535–2548. <https://doi.org/10.1113/jphysiol.2014.271387>
- Sin TK, Yu AP, Yung BY, Yip SP, Chan LW, Wong CS, Rudd JA, Siu PM (2015) Effects of long-term resveratrol-induced SIRT1 activation on insulin and apoptotic signalling in aged skeletal muscle. *Acta Diabetol* 52(6):1063–1075. <https://doi.org/10.1007/s00592-015-0767-3>
- Suofu Y, Li W, Jean-Alphonse FG, Jia J, Khattar NK, Li J, Baranov SV, Leronni D, Mihalik AC, He Y, Cecon E, Wehbi VL, Kim JH, Heath BE, Baranova OV, Wang X, Gable MJ, Kretz ES, Di Benedetto G, Lezon TR, Ferrando LM, Larkin TM, Sullivan M, Yablonska S, Wang J, Minnigh MB, Guillaumet G, Suzenet F, Richardson RM, Poloyac SM, Stolz DB, Jockers R, Witt-Enderby PA, Carlisle DL, Vilaradaga J-P, Friedlander RM (2017) Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc Natl Acad Sci USA* 114(38):E7997–E8006. <https://doi.org/10.1073/pnas.1705768114>
- Tan D, Chen L, Poeggeler B, Manchester L, Reiter R (1993) Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocr J* 1:57–60
- Tan D-X, Reiter RJ (2019) Mitochondria: the birth place, battle ground and the site of melatonin metabolism in cells. *Melatonin Res* 2(1):44–66. <https://doi.org/10.32794/mr11250011>
- Venkateshappa C, Harish G, Mahadevan A, Sirinivas Bharath MM, Shankar SK (2012) Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's Disease. *Neurochem Res* 2012(37):1601–1614. <https://doi.org/10.1007/s11064-012-0755-8>
- Wang F, Nguyen M, Qin FX-F, Tong Q (2007) SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell* 6(4):505–514. <https://doi.org/10.1111/j.1474-9726.2007.00304.x>
- Wang X, Sirianni A, Pei Z, Cormier K, Smith K, Jiang J, Zhou S, Wang H, Zhao R, Yano H, Kim JE, Li W, Kristal BS, Ferrante RJ, Friedlander RM (2011) The melatonin MT1 receptor axis modulates mutant Huntingtin-Mediated Toxicity. *J Neurosci* 31(41):14496–14507. <https://doi.org/10.1523/JNEUROSCI.3059-11.2011>
- Wang Xinkun, Michaelis EK (2010) Selective neuronal vulnerability to oxidative stress in the brain. *Front Aging Neurosci* 2(MAR):1–13. <https://doi.org/10.3389/fnagi.2010.00012>
- Weinreb O, Badinter F, Amit T, Bar-Am O, Youdim MBH (2015) Effect of long-term treatment with rasagiline on cognitive deficits and related molecular cascades in aged mice. *Neurobiol Aging* 36(9):2628–2636. <https://doi.org/10.1016/j.neurobiolaging.2015.05.009>
- Xu YZ, Deng XH, Bentivoglio M (2007) Differential response of apoptosis-regulatory Bcl-2 and Bax proteins to an inflammatory challenge in the cerebral cortex and hippocampus of aging mice. *Brain Res Bull* 74(5):329–335. <https://doi.org/10.1016/j.brainresbull.2007.07.002>
- Yanik M, Erel O, Kati M (2004) The relationship between potency of oxidative stress and severity of depression. *Acta Neuropsychiatrica* 16:200–203
- Zhao Y, Zhao R, Wu J, Wang Q, Pang K, Shi Q, Gao Q, Hu Y, Dong X, Zhang J, Sun J (2018) Melatonin protects against A β -induced neurotoxicity in primary neurons via miR-132/PTEN/AKT/FOXO3a pathway. *BioFactors* 44(6):609–618. <https://doi.org/10.1002/biof.1411>