Aminoguanidine ameliorates ovariectomy-induced neuronal deficits in rats by inhibiting AGE-mediated Aβ production

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Advanced glycation end products (AGEs) have been reported to cause neurodegeneration, senile plaque formation and spatial learning and memory deficits. There is much evidence describing the beneficial effects of aminoguanidine (AG) on the central nervous system; AG is able to inhibit the receptor for AGEs and beta-amyloid (Aβ) deposition in the brain, thus preventing cognitive decline and neurodegeneration. In this study, we investigated whether AG protects against ovariectomy-induced neuronal deficits and Aβ deposition in rats. Animals in the ovariectomy group (OVX) group, and those in the OVX+AG group were treated with AG (100 mg/kg/day) for 8 weeks. Learning and memory were evaluated using the electric Y maze. AGE and $Aβ_{1-40}$ biochemical assessments were performed using enzyme-linked immunosorbent assay (ELISA) kits. Furthermore, evaluations of brain amyloid precursor protein 695 (APP_{695}) mRNA expression by RT-PCR and AGE expression by immunohistochemistry were carried out. Ovariectomized rats exhibited memory impairment and Aβ production disorder with upregulated APP_{695} mRNA and AGE expression levels. AG pretreatment relieved the ovariectomy-induced learning and memory disorder and significantly ameliorated the Aβ production disturbance and AGE generation. Additionally, pathological changes in morphology were also significantly recovered. Our data reveal that AG plays a potentially neuroprotective role against ovariectomy-induced learning and cognitive impairment and Aβ production disorder.

Key words: aminoguanidine, ovariectomy, neuronal deficits, advanced glycation end products

INTRODUCTION

In 2019 Alzheimer's disease International (ADI) estimates that there are more than 50 million people are suffering from dementia globally, and the number is predicted soar to 152 million by 2050 (Alzheimer's Disease International, 2019). Increasing evidence has indicated that the oestrogen decline after menopause may influence learning and cognitive function, thus increasing the risk of Alzheimer's disease (AD) (Fukuzaki et al.,

2008). So far, the characteristic pathogenesis of AD included the deposition of beta-amyloid (Aβ), forming senile plaques and neurofibrillary tangles, disrupting synaptic plasticity and causing neuronal loss (Chen, 2018).

Recently, growing evidence has suggested that advanced glycation end products (AGEs) participate in the pathological processes of AD, including neurotoxicity and the aggregation of Aβ (Lubitz et al., 2016). AGEs are formed by the Maillard reaction and have been regarded as a primary source of neurotoxicity in Alzheimer's disease, which is affected by APP processing and Aβ forma-

tion *via* oxidative stress, resulting in the upregulation of cell apoptosis-related signalling pathways (Ko et al., 2015). However, preclinical and animal studies have revealed that AGEs mediate the estrogens dysfunction that occurs during onset of AD and participate in cognitive damage in peri- and post-menopausal women (Merhi, 2014).

Since a cohort study demonstrated that delayed hormone therapy after menopause accelerated the progression of dementia in women, researchers have attempted to discover the relationship between oestrogen and AD (Merlo et al., 2017). Accumulating evidence suggests that oestrogen has a protective effect against the pathogenesis of AD by regulating Aβ accumulation, inhibiting Aβ-mediated neurotoxicity, and enhancing Aβ clearance (Grimm et al., 2012). States of abnormal oestrogen levels during ageing are likely to cause aberrant astrogliosis and neuroinflammation-related neuronal cell death and interrupt cell-cell interactions (Yun et al., 2018). Sarkar et al. (2015) discovered that the oestrogen-induced amelioration of Aβ-induced damage in mitochondria is regulated by mitochondrial signalling pathways and oxidative phosphorylation involving estrogen receptor (ER) β and A kinase anchoring protein (AKAP). Further research suggested that oestrogen may protect against learning and memory impairment through the regulation of Aβ deposition and neuroinflammation by inhibiting nuclear factor kappa B (NF-κB) activity in both neuroglial and neuronal cells (Yun et al., 2018).

Aminoguanidine (AG), an AGE inhibitor, is a low-molecular-weight compound that exhibits selective suppression of inducible nitric oxide synthase (iNOS) and scavenges reactive oxygen species (ROS) (Pintus et al., 2018). AG is considered to have diverse pharmacological activities, such as the ability to repair tissue damage, prevent brain injury and stroke, and improve spinal cord motor function (Pearse et al., 2003; Di et al., 2008). It also inhibits Aβ-induced nuclear factor (NF)-κB p65 from translocating into the cytosol and prevents Aβ-induced neurological disorders through reducing the expression of iNOS and Cyclooxygenase-2 (COX-2) and inactivating NF-κB (Chen et al., 2017). To summarize, due to the anti-inflammatory and neuromodulatory advantages of AG, we considered it worthwhile to investigate the effects of AG on oestrogen deficiency-induced learning and cognitive impairment and Aβ production mediated by AGEs, which have not yet been reported.

In the present study, to verify the hypothesis and deduce the underlying mechanisms, a rat model of ovariectomy-induced neuronal deficits and Aβ deposition was established. The effects of AG on memory impairment, Aβ production and the mRNA expression levels of APP and AGEs in tissues, serum and urine were detected. In addition, to investigate the effects of AG on morphological changes in the cerebral cortex and hip-pocampus, paraffin sections were observed after being stained with haematoxylin and eosin (HE).

METHODS

Animals and reagents

This study was approved by the Hebei Provincial Hospital of Traditional Chinese Medicine Ethics Committee and all the experiments were carried out at research institute of this hospital. Adult female SD rats weighing 250-300 g (SPF grade) were supplied by the Experimental Animal Center of Beijing University of Chinese Medicine, China (license number SYXK (Beijing) 2012-0001). The animal studies abided by the guidelines established by the Chinese Committee on Experimental Animal Supervision. The study was a total of 36 rats, after one week of adapted to the lab conditions, animals were randomly divided into three experimental groups, with each group containing 12 rats. The rats were housed 3 per cage in transparent polysulfone cages (59 × 38 × 20 cm) containing wood-chip bedding and nesting material. The animals were maintained on standard laboratory diet and water *ad libitum*, and were housed under controlled conditions of temperature (23±2°C), humidity (50±10%), air system filtration (10-20 ventilations/hour) and on a 12 h : 12 h light : dark cycle.

The rats were randomly assigned to one of the three following groups: the sham group (sham); the ovariectomy group (OVX); and the aminoguanidine group (OVX+AG). Animals in the OVX group underwent bilateral ovariectomy, and those in the sham group underwent sham surgery. Four weeks after the surgery, animals in the OVX group were randomly assigned to one of two groups, with ten animals in each group (four rats died). Animals in OVX group underwent gavage with saline, those in the OVX+AG group was administered an aqueous solution of AG, and those in the sham group were also infused with saline. Finally, rats in each experimental group (n=10-12) were studied. AG (Sigma, St. Louis, MO, USA) was dissolved thoroughly in sterile water and given to the animals every morning at 09:00 a.m. The 0.1% AG dose delivered to each animal was calculated as 100 mg/kg/day for 8 weeks (Díaz et al., 2014). Then the rats were tested using the electric Y maze.

Electric Y maze

The spatial learning and memory of rats were evaluated using the electric Y maze. The test was executed as explained by Amat et al. (2012) with subtle alterations. Briefly, the electric Y maze consisted of a con-

ductive grid floor with three identical arms (40 L × 10 W × 20 H cm) placed at 120° relative to each other; the arms were made of dark plexiglas. Two arms were unsafe zones, while the other was a safe zone. The rats were tested for two consecutive days; the correct escape rate was determined by the number of passes into the unsafe zones within ten repetitions, and the escape latency was determined by the time to the first pass into the safe zone from an unsafe zone.

ELISA

After the behavioural evaluation, all survived animals were humanely sacrificed at the 21th week of the experiment by an intraperitoneal injection of 5% chloral hydrate (0.4 mL/kg). The urine was collected and blood was sampled from the abdominal artery, and both samples were stored at -80°C. Then, the rats were sacrificed and the brain was excised. One side of the hippocampus and cerebral cortex was rapidly stored at -80°C for future use, while the other side was fixed with 4% paraformaldehyde. The $A\beta_{1-40}$ levels in the hippocampus and cerebral cortex were determined using ELISA kits (AnaSpec, USA) according to the manufacturer's instructions. The AGE levels were determined using ELISA according to the method by Vitek et al., 1994. Briefly, minced hippocampus and cerebral cortex tissues were dissolved in PBS (phosphate-buffered saline; 0.01 mol/L, pH 7.0-7.2), and the homogenates were centrifuged for 20 min at 5000 g. Then, the resultant suspension (100 ml) was added to a 96-well plate, 100 μL/well, covered with the appropriate purified AGEs (1:2000) and $A\beta_{1-40}$ (1:1000) antibody and incubated for 120 min at 37°C. The standard curve of AGEs and $A\beta_{1-40}$, at a concentration of 0.05~400 μg/mL and 970~11,285 pg/mL, respectively. The samples in the 96-well plate were rapidly measured at 450 nm using a microplate reader (Tecan, Switzerland). After three repeated measurements, the mean and standard deviation were calculated for the tested samples.

Fluorescence measurements

Additionally, the serum and urine AGE levels were quantified using immunofluorescence technique based on the Edelstein's protocol (Edelstein and Brownlee, 1992). The serum and urine were centrifuged for 20 min at 3000 g, solution was diluted at 1:10 by saline solution. The fluorescence intensity of AGEs in serum and urine was obtained at 380 nm excitation and 420 nm emission wavelengths using a spectrophotometer. The excitation and emission bandwidths were 5 and 10 nm, respectively. Fluorescent AGE (Fluo-AGE) levels are expressed in arbitrary units. Moreover, the ELISA for AGE detection preferentially quantifies nonfluorescent AGEs (N(ε)-carboxymethyllysine (CML)) (Rondeau and Bourdon, 2011). The solution was centrifuged for 20 min at 3000 g, serum was diluted at 1:400, and urine was diluted at 1:10. The other steps were the same as those described above.

Reverse transcriptase polymerase chain reaction (RT-PCR)

The RNA of APP_{695} in the hippocampus and cerebral cortex was refined using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). APP_{695} and glyceraldehyde-phosphate dehydrogenase (GAPDH) mRNA expression levels were analysed by RT-PCR. RT-PCR was performed using a reverse transcriptase kit (Promega Corporation, Madison, WI, USA). All primers were designed and synthesized by Shanghai Sangon Biotech, China. The primer sequences are shown in Table I. FluorChem FC2 software (Alpha Innotech, CA, USA) was used to photo and analyze the gray value of the mRNA expression in each group. The RT-PCR protocol conformed to that reported in our previous study (Xi et al., 2012).

Histological and immunohistochemical methods

The hippocampus and cerebral cortex were fixed with 10% formalin solution and then embedded in paraffin. The paraffin blocks were sectioned at 5 μm and stained with haematoxylin and eosin (HE). The stained tissues were observed using a Leica DM-LS microscope at 40× and 400× magnification. Immunohistochemical staining was performed as previously described (Hwang et al., 2015). At the end of the study, the brain was fixed in 4% paraformaldehyde for 24 h, soaked in 70% ethanol and embedded in paraffin. The sections (20 μm) of the hippocampus and cerebral cortex were

Table I. Primer sequences and annealing temperature.

Primer	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Annealing temperature (°C)
GAPDH	CCCACGGCAAGTTCAACGGCA	TGGCAGGTTTCTCCAGGCGGC	59
APP_{695}	GACTCCGATGTCTGGTGGGG	TGTCAGCTTTGGGCAAATTCTT	58

immunostained using a peroxidase anti-peroxidase method with DAB as the chromogen. Primary antibody incubation was performed using anti-AGE antibody (Abcam, Cambridge, (MA) USA) at a 1:500 dilution overnight at 4°C. After rinsing with phosphate-buffered saline (PBS), a secondary rabbit anti-rat antibody Invitrogen, USA was added, as was diaminobenzidine (DAB) for colour development. The AGE expression levels in the brain were assessed based on the greyscale density per high-power field (HPF) determined using image analysis software (Leica QWin Standard V 2.8, UK). Photomicrographs were obtained using a digital camera system (AxioCam Imager; Carl Zeiss) and a confocal laser scanning microscope at 400× magnification.

Statistical analysis

All data are shown as the mean ± standard deviation (SD) for all experiments. Differences between groups were analysed using the Statistical Package for Social Science (SPSS) 22.0 (SPSS, Inc., Chicago, USA) software package and one-way analysis of variance (ANOVA) followed by SNK or scheffé's multiple comparison test. All statistical analyses were 2-sided, and significant differences were considered at $P<0.05$.

RESULTS

Effects of AG on ovariectomy-induced memory impairment in rats

To explore the effects of AG on spatial learning and memory impairment, rats in each experimental group (n=10-12) were assessed using the Y maze task. Rats in the OVX group exhibited a remarkably reduced correct escape rate compared with those in the sham group ($P<$ 0.01), and an increasing rate was observed in the OVX+AG group compared with the OVX group (F=5.162; df=2; P=0.014) (Fig. 1A). Rats in the OVX+AG group showed a significantly shorter escape latency than rats in the sham and OVX groups (F=3.842; df=2; P=0.036) (Fig. 1B).

Effects of AG on ovariectomy-induced Aβ production in rats

Compared with the sham group, the $Aβ_{1-40}$ content in the cerebral cortex was increased in the OVX group (F=3.134 df=2; P=0.062); moreover, the $Aβ_{1-40}$ content in the hippocampus exhibited no significant changes (P=0.123). After treatment with AG, the $Aβ_{1-40}$ content in the cerebral cortex (P<0.01) and hippocampus (P<0.05)
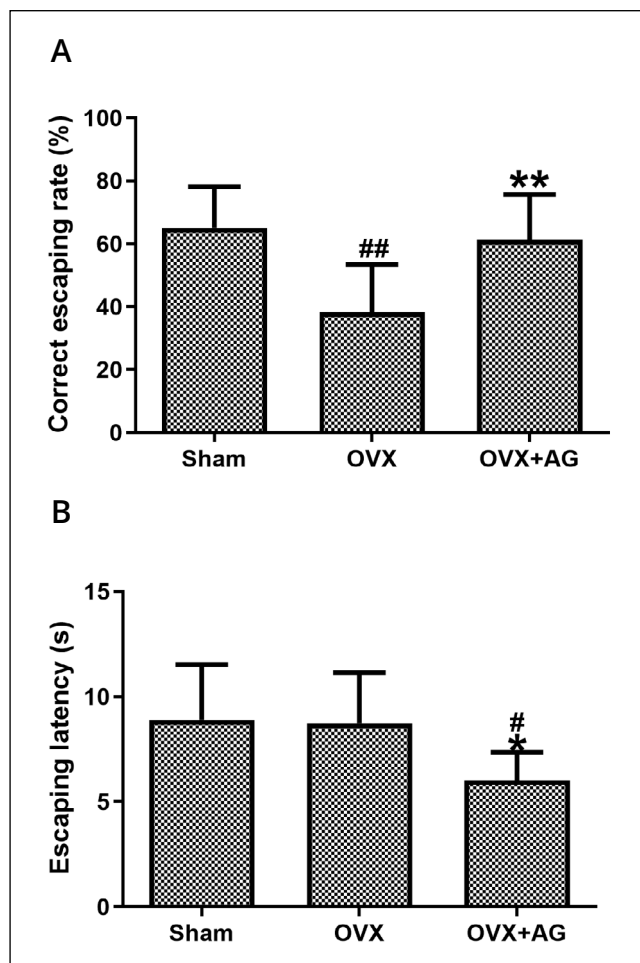

Fig. 1. Effects of AG on ovariectomized rats learning and memory change. (A) Correct escaping rate, (B) Escape latency were measured using the Y-electric maze. All data are shown as mean ± SD (n=10-12 per group). #P<0.05 compared with the sham group; ##P<0.01 compared with the sham group. *P<0.05 compared with the OVX group; **P<0.01 compared with the OVX group.

was significantly diminished compared with after OVX (F=5.290; df=2; P=0.008) (Fig. 2).

As shown in Fig. 3, APP_{695} mRNA expression was upregulated in the OVX group in both the cerebral cortex and hippocampus compared with that in the sham group (P<0.01). However, AG remarkably downregulated APP_{695} mRNA expression in the cerebral cortex and hippocampus compared with OVX (P<0.01) (Fig. 3).

Effects of AG on ovariectomy-induced AGE changes

The AGEs contents of the cerebral cortex and hippocampus in the OVX group were showed not significantly different from those in the sham group (P>0.05). After treatment with AG, the AGE content in the cerebral

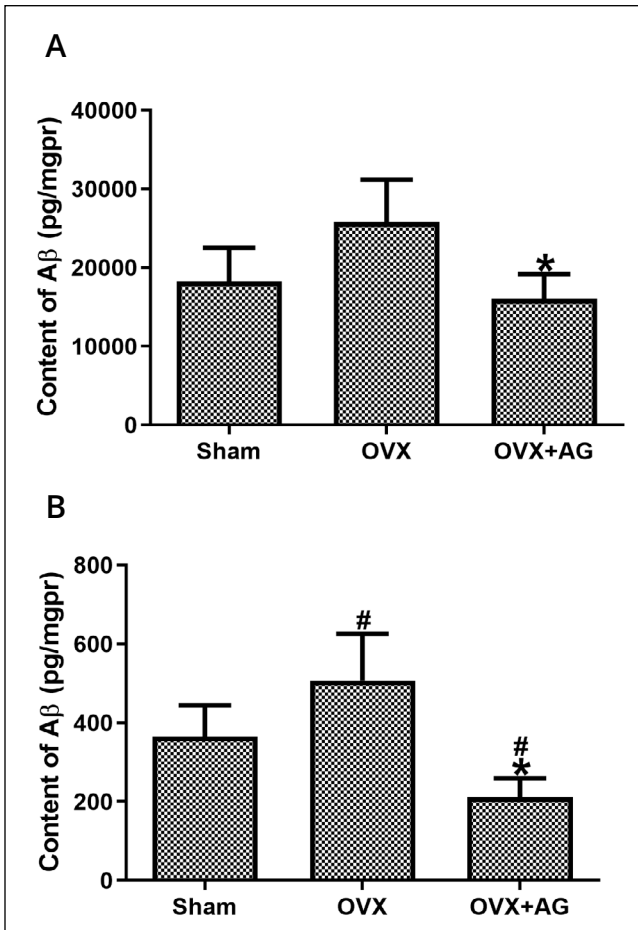Fig. 2. Contents of Aβ in the hippocampus (A) and cerebral cortex (B) in each group. All data are shown as mean ± SD (n=10-12 per group). #$P<0.05$ compared with the sham group; ##$P<0.01$ compared with the sham group. *$P<0.05$ compared with the OVX group; **$P<0.01$ compared with the OVX group.

cortex significantly declined (F=3.053; df=2; $P=0.039$) (Fig. 4A).

The AGE contents in the serum were significantly increased in the OVX group compared with the sham group (F=4.046; df=2; $P=0.03$), but no significant difference in the urine AGE contents was found between the two groups ($P>0.05$). Additionally, the urine AGE levels were significantly increased after treatment with AG compared to after the sham and OVX treatments (F=5.334; df=2; $P=0.013$). However, the two methods for detecting the serum AGE levels in the OVX+AG group showed different results, with one level less than that in the OVX group ($P<0.01$) and the other significantly greater than that in the sham group (F=5.698; df=2; $P=0.002$) (Table II).

As shown in Fig. 4B, immunohistochemical staining indicated that AGE expression was significantly elevated in the OVX group compared with the sham group ($P<0.05$). Moreover, the AGE levels were significantly lower in the OVX+AG group than in the OVX group ($P<0.05$).
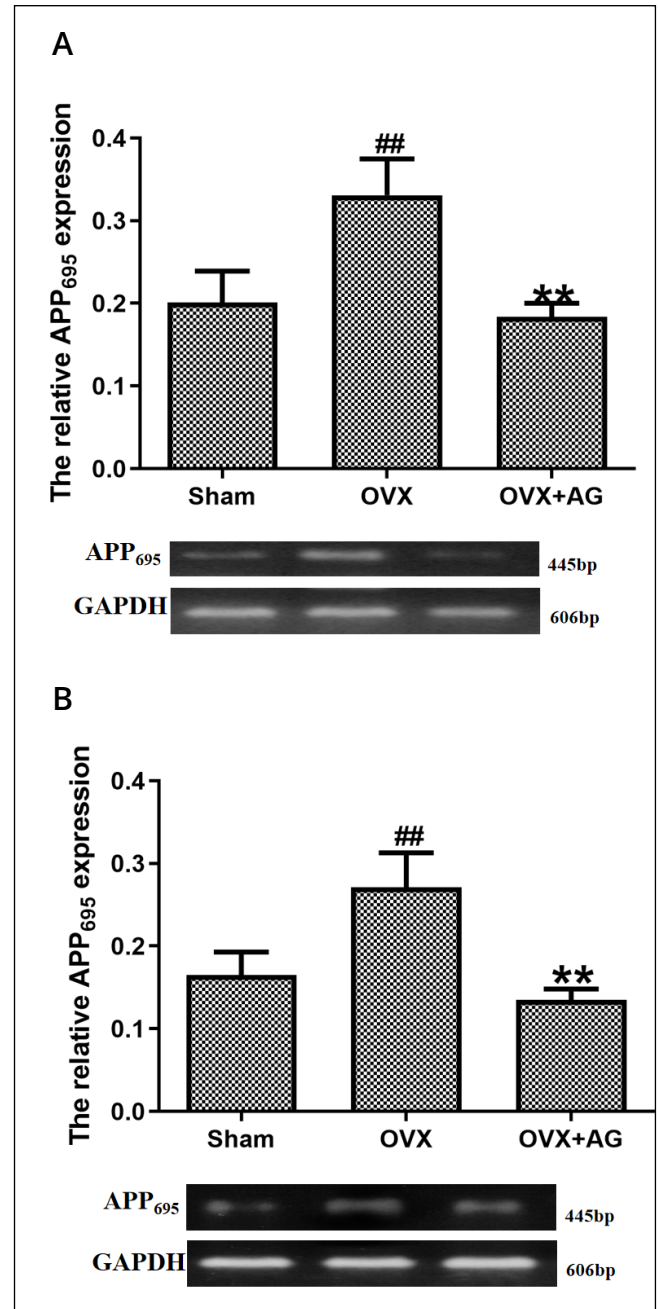

Fig. 3. The mRNA expressions of AGEs in the hippocampus (A) and cerebral cortex (B) in each group by RT-PCR. All data are shown as mean ± SD (n=10-12 per group). #$P<0.05$ compared with the sham group; ##$P<0.01$ compared with the sham group. *$P<0.05$ compared with the OVX group; **$P<0.01$ compared with the OVX group.

Effect of AG on ovariectomy-induced morphological changes in the rat cerebral cortex and hippocampus

In the sham group, the structures of the cerebral cortex and hippocampus were normal, the nucleus and
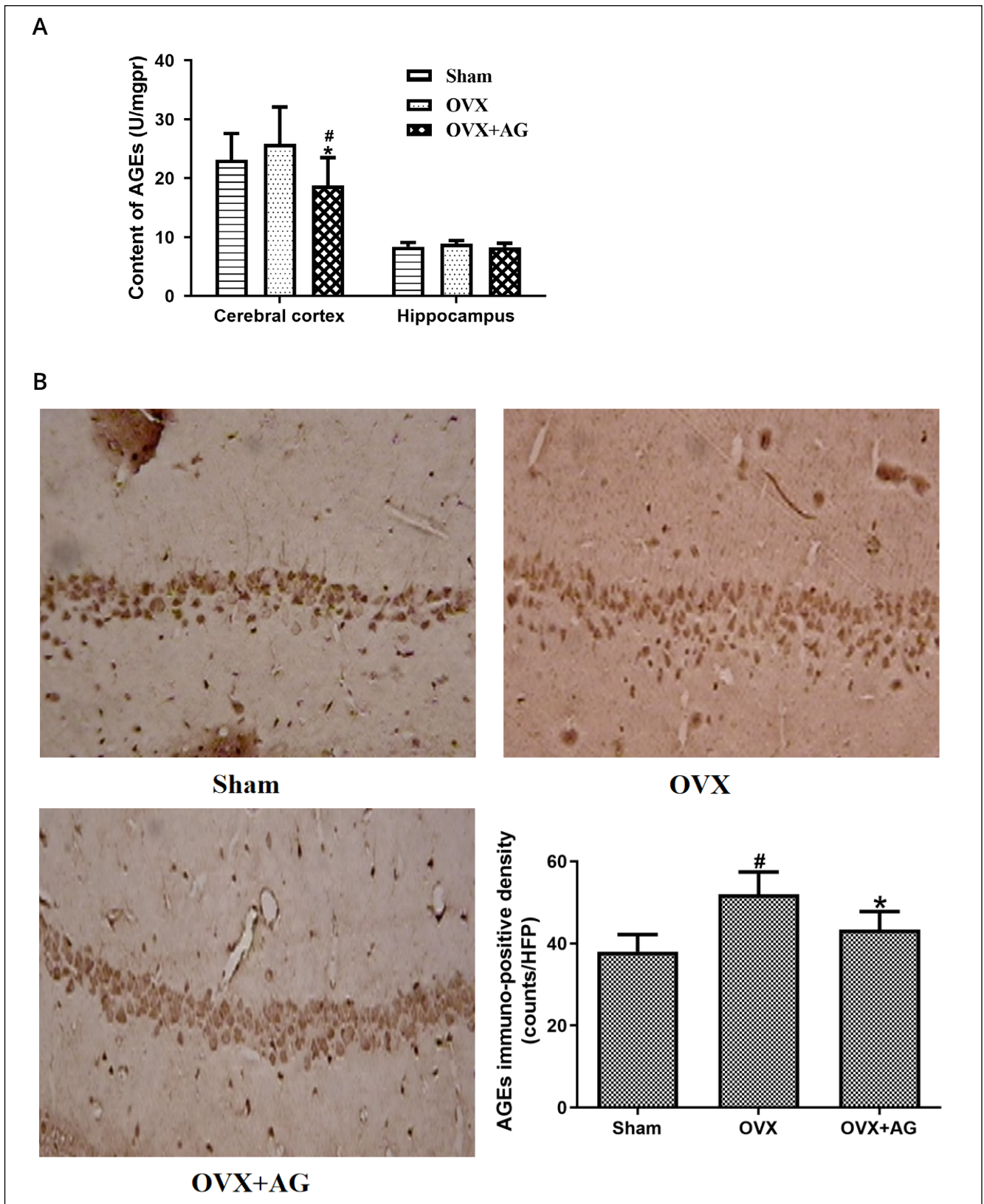
Fig. 4. Levels of AGEs in the hippocampus and cerebral cortex of the studied groups (A). The AGEs immune-positive expression in brain tissues of each group (B). All data are presented as mean ± SD (n=10-12 per group). #P<0.05 compared with the sham group; ##P<0.01 compared with the sham group. *P<0.05 compared with the OVX group; **P<0.01 compared with the OVX group.

Table II. Levels of AGEs in serum and urine ($\bar{x}$±s, n=10-12).

Groups	Fluorescent AGEs		Anti-CML AGEs	
	Serum (AU/mgpr)	Urine (AU/mgpr)	Serum (U/ml)	Urine (U/gpr)
Sham	1.26±0.04	2641.10±425.42	3.93±0.60	11.82±2.14
OVX	1.73±0.35[#]	2429.11±550.83	4.84±0.67[#]	13.60±2.48
OVX+AG	1.22±0.27**	3709.20±863.7*[#]	5.27±0.84[##]	17.91±3.69*[#]

[#] *P*<0.05 *vs.* sham, [##] *P*<0.01 *vs.* sham; * *P*<0.05 *vs.* OVX, ** *P*<0.01 *vs.* OVX.

membrane of neural cells exhibited integrity, and the hippocampal pyramidal cells were aligned in an orderly manner. In contrast, in the OVX group, the cerebral cortex and hippocampus showed diffuse shrinkage, widened sulci and enlarged ventricles; additionally, vacuoles in the neurons and reduced cell membrane integrity were identified. In the OVX+AG group, the pathological changes in morphology observed in the OVX group were significantly recovered (Fig. 5).

DISCUSSION

Until now, the pathogenic process that causes AD has not been fully understood; however, several genetic and biochemical factors, such as APP mutations, Aβ deposition, tau protein hyperphosphorylation, inflammation, oestrogen deficiency, oxidative stress and glycation, contribute to AD pathogenesis (Parihar and Hemnani, 2004). In recent decades, the neuroprotective effects of estrogens against neuroinflammatory and neurodegenerative aberrations have been well reported by numerous researchers (Engler-Chiurazzi et al., 2017). Clinical trials have shown that the incidence of AD in women is 2-3 times higher than that in men, and the completion of menopause significantly increases the risk of cognitive impairment (Pike, 2017). A recent study using a rodent model showed that reduced 17β-oestradiol levels were related to cognitive impairment in ovariectomized rats, and further research suggested that a 17β-oestradiol prodrug could effectively reduce Aβ levels (Tschiffely et al., 2016). In addition, estrogens not only affect synaptotoxicity, oxidative stress and neuroinflammation but also directly affect neurons, as well as neuronal cells, astrocytes and microglia selectively (Ben Halima et al., 2016). AD-associated decreases in estrogens and the resulting morphological changes were observed in ovariectomized rats in our study. HE staining of cerebral cortex and hippocampal sections showed diffuse shrinkage, widened sulci and enlarged ventricles, as well as vacuoles in neurons and reduced cell membrane integrity in the OVX group.

The results of the present study indicate that rats in the OVX group exhibited significant learning and cognitive impairment compared with those of rats in the sham group. We examined the effects of AG in rats with ovariectomy-induced memory impairment using the Y maze, which indicated the spatial learning ability and working memory of the rats. We observed that AG improved both spatial learning and memory, as evidenced by reductions in the escape latency and significant increases in the correct escape rate in the Y maze test. Our results were consistent with those of previous reports showing that ovariectomy led to spatial learning and memory impairment. Zakeri et al. (2019) suggested that ovariectomy influenced anxiety-like behaviour, working memory, and physical strength through the elevated plus maze, Y maze and swimming capacity test. Sarkaki et al. (2008) indicated that OVX rats exhibited impaired performance in locating a hidden escape platform in the Morris water maze. In addition, Snihur et al. (2008) showed that OVX adult rats exhibited impaired spatial navigation in the Morris water maze, as measured by search time and direct and circular swim persistence times. However, our results revealed that oestrogen deprivation (OVX group) had no effect on the escape latency, while it impaired the correct escape rate. Even though this result was inconsistent with previously published findings, numerous reasons could be offered to explain these discrepancies. Djiogue et al. (2018) observed that ovariectomy had no significant effects on short-term memory, indicating an indirect link between ovariectomy and long-term memory effects. Yamada et al. (1999) reported that neither long-term (3 months) nor short-term (1 month) deprivation of oestrogen by ovariectomy caused significant impairments in spatial learning and memory in the water maze or spontaneous alteration behaviours in the Y-maze. This finding suggests that short-term memory depends more on specific areas in the brain, while long-term memory relies on hippocampal formation in mammals and that the oestrogen effects are area-specific and time-of-duration-specific in the brain (Zhao et al., 2012; Kim and Frick, 2017). Thus, future studies
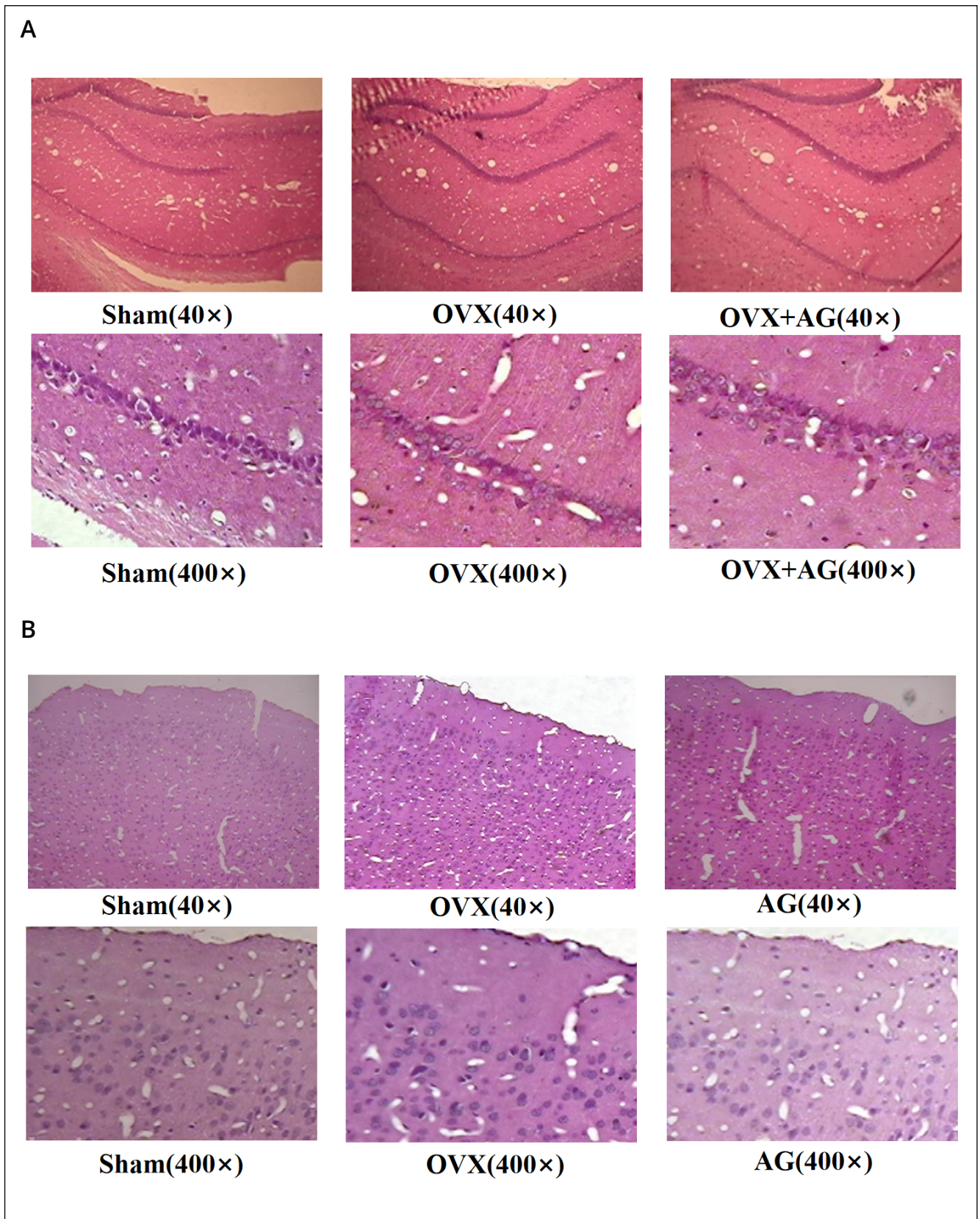
Fig. 5. HE staining was used in paraffin-embedded sections of the hippocampus (A) and cerebral cortex (B) in each group (HE staining, light microscope, ×40, ×100 and ×400).

attempting to confirm the available data should consider multiple factors, including the specific area in the brain and experimental time of duration.

Aminoguanidine, a well-known AGE inhibitor, is a nucleophilic hydrazine compound that prevents AGE formation. AG has been reported to inhibit inflammatory reactions and reduce potential neurodegeneration in numerous experimental dementia models (Rodrigues et al., 2009). It has also been suggested that AG prevents cognitive impairment and reduces glial activation in the brain of mice with dementia induced by streptozotocin. Additionally, the beneficial effects of AG on the CNS include the suppression of AGE receptors in the brain, thus preventing learning and cognitive function decline and Aβ deposition in mice subjected to transverse aortic coarctation (TAC) (Carnevale et al., 2012). The present study aimed to illuminate the possible mechanisms underlying the neuroprotective effects of AG, such as the inhibition of ovariectomy-induced neuronal deficits in rats via the inhibition of AGE-mediation modulation of Aβ production. Ovariectomized rats can be considered models that imitate many cellular and molecular changes in neurodegenerative diseases, such as AD and Parkinson's disease. Simultaneously, decreasing estrogens levels might be followed by Aβ accumulation and hippocampal volume loss, which in turn play key roles in neurodegeneration (Mosconi et al., 2018).

Several observations suggest that AD is characterized by toxic Aβ produced by β-secretase (BACE1)-mediated cleavage of APP, and the accumulation of Aβ might trigger the pathological activation of APP signalling, leading to neuronal dysfunction (Lan et al., 2015; Bignante and Lorenzo, 2018). APP, a precursor of Aβ that also binds Aβ fibrils, mediates neurotoxic effects on neuronal growth and subsequently extends, resulting in diverse Aβ assemblies. Moreover, there have been reports demonstrating that Aβ-derived ligands and Aβ oligomers extracted from the human brain in AD impair long-term potentiation and partially accelerate neurofibrillary tangle (NFT) formation (Fukuzaki et al., 2008). In this study, ovariectomized rats showed increased levels of APP_{695} mRNA expression and significantly increased $A\beta_{1-40}$ levels in the cerebral cortex. The increased expression levels of APP and Aβ can partially account for the learning and memory impairments observed in these rats. AG (100 mg/kg/day) significantly decreased the levels of $A\beta_{1-40}$ and APP_{695} mRNA expression. Our results are consistent with those of other researchers who verified that AG inhibits Aβ-induced neurological disorders. However, we also found that OVX did not affect hippocampal Aβ1-40 levels. Consistent with our findings, several studies have demonstrated that

serum Aβ1-40 levels were elevated in OVX animal models, although there was no effect on hippocampal Aβ1-40 levels (Wang et al., 2017). These findings suggest that brain Aβ accumulation depends on not only ovarian hormonal decline but also complex regulation in the brain.

An increasing number of studies have shown that ovariectomy *in vivo* causes learning and memory impairments through the modulation of Aβ deposition, neuroinflammation and other mechanisms (Yun et al., 2018). There is also growing evidence that AGEs may affect the neuropathological and biochemical features of neurodegenerative processes, such as increased protein cross-linking, oxidative stress, and neuronal death (Uribarri et al., 2015). *In vivo*, AGEs are combined with deposits of proteins, such as Aβ, resulting in plaque formation in the brain. In an AD-like animal model, a high-AGEs diet caused significant learning and memory impairments, insoluble Aβ42 and AGEs assembly in the hippocampus, and increased levels of oxidative stress. Lubitz et al. (2016) also reported that the AGEs receptor binds to Aβ42 and affects its transport across the blood-brain barrier. Ko et al. (2010) demonstrated that AGEs regulate APP and APP synthesis pathways via ROS and enhance the deposition of Aβ. It is known that the soluble isoform binds to ligands and prevents the negative effects of receptor activation. Walker et al. (2015) demonstrated cognitive impairment, upregulated Aβ production, neurotoxicity and inflammation in the neurons or microglia of transgenic mice overexpressing the receptor for AGEs (RAGE). Similarly, the ovariectomized rats in our study also showed upregulated APP, Aβ and AGEs levels, which caused spatial memory degeneration and morphological changes in the cerebral cortex and hippocampus. AG is itself a potent inhibitor of RAGE in the brain and thus prevents cognitive impairment and Aβ aggregation (Ben Halima et al., 2016). Our study shows that AG pretreatment prevents the memory deficits induced by ovariectomy because rats in the OVX+AG group demonstrated a reduced AGEs levels in the cerebral cortex and few morphological changes in the brain tissue, but hippocampal AGEs levels were not significantly changed (Fig. 4). Few studies have investigated the mechanisms by which AG promotes OVX-induced neuronal deficits in rats. The currently-accepted hypothesis is that AG mainly modulates Aβ production by inhibiting AGEs. However, the mechanisms are unclear, and the complexity of brain-neural functions is unclear. Thus, further studies are required to confirm these findings in the context of integrating multiple factors, including the specific sites of action in the brain, experimental durations, and other potential reasons involved.

CONCLUSION

In conclusion, our data reveal that AG relieved ovariectomy-induced learning and memory impairment in the hippocampus and cortex of rats. In our study, the neuroprotective effects of AG can be attributed to its anti-AGEs activity and its ability to reduce the levels of Aβ and its precursor protein APP. However, further study is needed to determine the exact pathways and specific sites of action responsible for these effects.

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AUTHORS' CONTRIBUTIONS

Y.F.W designed the study, prepared the animal ethics and funding applications, D.D.Z drafted and revised the manuscript, Y.G.W helped in animal ethics and performed the statistical analyses, C.Y.L and Z.H.W helped in analysis of the data and preparation of the manuscript and conducted and designed the animals studies.

REFERENCES

Alzheimer's Disease International (2019) World Alzheimer Report 2019: Attitudes to dementia. London (GB): ADI.

Amat N, Hoxur P, Ming D, Matsidik A, Kijjoa A, Upur H (2012) Behavioral, neurochemical and neuroendocrine effects of abnormal savda munziq in the chronic stress mice. Evid Based Complement Alternat Med 2012: 426757.

Ben Halima S, Mishra S, Raja KMP, Willem M, Baici A, Simons K, Brüstle O, Koch P, Haass C, Caflisch A, Rajendran L (2016) Specific inhibition of β-secretase processing of the Alzheimer disease amyloid precursor protein. Cell Rep 14: 2127–2141.

Bignante EA, Lorenzo A (2018) APP signaling in Alzheimer's disease. Aging 10: 3063–3064.

Carnevale D, Mascio G, D'Andrea I, Fardella V, Bell RD, Branchi I, Pallante F, Zlokovic B, Yan SS, Lembo G (2012) Hypertension induces brain b-amyloid accumulation, cognitive impairment, and memory deterioration through activation of receptor for advanced glycation end products in brain vasculature. Hypertension 60: 188–197.

Chen YG (2018) Research progress in the pathogenesis of Alzheimer's disease. Chin Med J 131: 1618–1624.

Chen T, Sun XL, Yang XA, Shi JJ, Liu Y, Gong JM (2017) Aminoguanidine exhibits an inhibitory effect on β-amyloid induced damage in F98 glioma cells. Mol Med Rep 16: 6116–6121.

Di F, Yan-Ting G, Hui L, Tao T, Zai-Hua X, Xue-Ying S, Hong-Li X, Yun-Jie W (2008) Role of aminoguanidine in brain protection in surgical brain injury in rat. Neurosci Let 448: 204–207.

Díaz A, Rojas K, Espinosa B, Chávez R, Zenteno E, Limón D, Guevara J (2014) Aminoguanidine treatment ameliorates inflammatory responses and memory impairment induced by amyloid-beta 25-35 injection in rats. Neuropeptides 48: 153–159.

Djiogue S, Djiyou Djeuda AB, Seke Etet PF, Ketcha Wanda GJM, Djikem Tadah RN, Njamen D (2018) Memory and exploratory behavior impairment in ovariectomized Wistar rats. Behav Brain Funct 14: 14.

Edelstein D, Brownlee M (1992) Mechanistic studies of advanced glycosylation end products inhibition by aminoguanidine. Diabetes 41: 26–29.

Engler-Chiurazzi EB, Brown CM, Povroznik JM, Simpkins JW (2017) Estrogens as neuroprotectants: estrogenic actions in the context of cognitive aging and brain injury. Prog Neurobiol 157: 188–211.

Fukuzaki E, Takuma K, Funatsu Y, Himeno Y, Kitahara Y, Gu B, Mizoguchi H, Ibi B, Koike K, Inoue M, Du-Yan S, Yamada K (2008) Ovariectomy increases neuronal amyloid-beta binding alcohol dehydrogenase level in the mouse hippocampus. Neurochem Int 52: 1358–1364.

Grimm A, Lim YA, Mensah-Nyagan AG, Götz J, Eckert A (2012) Alzheimer's disease, oestrogen and mitochondria: an ambiguous relationship. Mol Neurobiol 46: 151–160.

Hwang CJ, Yun HM, Park KR, Song JK, Seo HO, Hyun BK, Choi DY, Yoo HS, Oh KW, Hwang DY, Han SB, Hong JT (2015) Memory impairment in estrogen receptor alpha knockout mice through accumulation of amyloid-beta peptides. Mol Neurobiol 52: 176–186.

Kim J, Frick KM (2017) Distinct effects of estrogen receptor antagonism on object recognition and spatial memory consolidation in ovariectomized mice. Psychoneuroendocrinology 85: 110–114.

Ko SY, Ko HA, Chu KH, Shieh TM, Chi TC, Chen HI, Chang WC, Chang SS (2015) The possible mechanism of advanced glycation end products (AGEs) for Alzheimer's disease. PLoS One 10: e0143345.

Ko SY, Lin YP, Lin YS, Chang SS (2010) Advanced glycation end products enhance amyloid precursor protein expression by inducing reactive oxygen species. Free Radic Biol Med 49: 474–480.

Lan YL, Zhao J, Li S (2015) Update on the neuroprotective effect of estrogen receptor alpha against Alzheimer's disease. J Alzheimer's Dis 43: 1137–1148.

Lubitz I, Ricny J, Atrakchi-Baranes D, Shemesh C, Kravitz E, Liraz-Zaltsman S, Maksin-Matveev A, Cooper I, Leibowitz A, Uribarri J, Schmeidler J, Jing Cai W, Kristofikova Z, Ripova D, LeRoith D, Schnaider-Beeri M (2016) High dietary advanced glycation end products are associated with poorer spatial learning and accelerated Aβ deposition in an Alzheimer mouse model. Aging Cell 15: 309–316.

Merhi Z (2014) Advanced glycation end-products: pathway of potentially significant pathophysio-logical and therapeutic relevance for metabolic syndrome in menopausal women. J Clin Endocrinol Metab 99: 1146–1148.

Merlo S, Spampinato SF, Sortino MA (2017) Estrogen and Alzheimer's disease: Still an attractive topic despite disappointment from early clinical results. Eur J Pharmacol 817: 51–58.

Mosconi L, Rahman A, Diaz I, Wu X, Scheyer O, Hristov HW, Vallabhajosula S, Isaacson RS, de Leon MJ, Brinton RD (2018) Increased Alzheimer's risk during the menopause transition: A 3-year longitudinal brain imaging study. PLoS One 13: e0207885.

Parihar MS, Hemnani T (2004) Alzheimer's disease pathogenesis and therapeutic interventions. J Clin Neurosci 11: 456–467.

Pearse DD, Chatzipanteli K, Marcillo AE, Bunge MB, Dietrich WD (2003) Comparison of iNOS inhibition by antisense and pharmacological inhibitors after spinal cord injury. J Neuropathol Exp Neurol 62: 1096–1107.

Pike CJ (2017) Sex and the development of Alzheimer's disease. J Neurosci Res 95: 671–680.

Pintus E, Kadlec M, Jovičić M, Sedmíková M, Ros-Santaella JL (2018) Aminoguanidine protects boar spermatozoa against the deleterious effects of oxidative stress. Pharmaceutics 10: 212.

Rodrigues L, Biasibetti R, Swarowsky A, Leite MC, Quincozes-Santos A, Quilfeldt JA, Achaval M, Gonçalves CA (2009) Hippocampal alterations in rats submitted to streptozotocin-induced dementia model are prevented by aminoguanidine. J Alzheimers Dis 17: 193–202.

Rondeau P, Bourdon E (2011) The glycation of albumin: structural and functional impacts. Biochimie 93: 645–658.

Sarkar S, Jun S, Simpkins JW (2015) Estrogen amelioration of Aβ-induced defects in mitochondria is mediated by mitochondrial signaling pathway involving ERβ, AKAP and Drp1. Brain Res 1616: 101–111.

Sarkaki A, Amani R, Badavi M, Safahani M, Aligholi H (2008) Effect of ovariectomy on reference memory version of Morris water maze in young adult rats. Iran Biomed J 12: 123–128.

Snihur AW, Hampson E, Cain DP (2008) Estradiol and corticosterone independently impair spatial navigation in the Morris water maze in adult female rats. Behav Brain Res 187: 56–66.

Tschiffely AE, Schuh RA, Prokai-Tatrai K, Prokai L, Ottinger MA (2016) A comparative evaluation of treatments with 17beta-estradiol and its brain-selective prodrug in a double-transgenic mouse model of Alzheimer's disease. Horm Behav 83: 39–44.

Uribarri J, del Castillo MD, de la Maza MP, Filip R, Gugliucci A, Luevano-Contreras C, Macías-Cervantes MH, Markowicz Bastos DH, Medrano A, Menini T, Portero-Otin M, Rojas A, Sampaio GR, Wrobel K, Wrobel K, Garay-Sevilla ME (2015) Dietary advanced glycation end products and their role in health and disease. Adv Nutr 6: 461–473.

Vitek MP, Bhattacharya K, Glendening JM, Stopa E, Vlassara H, Bucala R, Manogue K, Cerami A (1994) Advanced glycation end products contribute to amyloidosis in Alzheimer's disease. Proc Natl Acad Sci 91: 4766–4770.

Walker D, Lue LF, Paul G, Patel A, Sabbagh MN (2015) Receptor for advanced glycation endproduct modulators: a new therapeutic target in Alzheimer's disease. Expert Opin Investig Drugs 24: 393–399.

Wang Z, Jackson RJ, Hong W, Taylor WM, Corbett GT, Moreno A, Liu W, Li S, Frosch MP, Slutsky I, Young-Pearse TL, Spires-Jones TL, Walsh DM (2017) Human brain-derived Aβ oligomers bind to synapses and disrupt synaptic activity in a manner that requires APP. J Neurosci 37: 11947–11966.

Xi YD, Yu HL, Ding J, Ma WW, Yuan LH, Feng JF, Xiao YX, Xiao R (2012) Flavonoids protect cerebrovascular endothelial cells through Nrf2 and PI3K from beta-amyloid peptide-induced oxidative damage. Curr Neurovasc Res 9: 32–41.

Yamada K, Tanaka T, Zou LB, Senzaki K, Yano K, Osada T, Ana O, Ren X, Kameyama T, Nabeshima T (1999) Long-term deprivation of oestrogens by ovariectomy potentiates beta amyloid-induced working memory deficits in rats. Brit J Pharmacol 128: 419–427.

Yun J, Yeo IJ, Hwang CJ, Choi DY, Im HS, Kim JY, Choi WR, Jung MH, Han SB, Hong JT (2018) Estrogen deficiency exacerbates Aβ-induced memory impairment through enhancement of neuroinflammation, amyloidogenesis and NF-κB activation in ovariectomized mice. Brain Behav Immun 73: 282–293.

Zakeri M, Fatemi I, Kaeidi A, Zakeri MA, Hakimizadeh E, Hassanipour M, Rahmani M, Hassanshahi J, Ayoobi F, Allahtavakoli M (2019) Pro-neurocognitive and anti-sarcopenic benefits of one-year metformin therapy in ovariectomized aged mice. Clin Exp Pharmacol Physiol 46: 1133–1140.

Zhao Z, Fan L, Fortress AM, Boulware MI, Frick KM (2012) Hippocampal histone acetylation regulates object recognition and the estradiol-induced enhancement of object recognition. J Neurosci 32: 2344–2351.