Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-200103
IOS Press

S-Adenosylmethionine Alleviates Amyloid-β-Induced Neural Injury by Enhancing Trans-Sulfuration Pathway Activity in Astrocytes

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Accepted 9 May 2020

Abstract.
Background: Glutathione (GSH) is an important endogenous antioxidant protecting cells from oxidative injury. Cysteine (Cys), the substrate limiting the production of GSH, is mainly generated from the trans-sulfuration pathway. S-adenosylmethionine (SAM) is a critical molecule produced in the methionine cycle and can be utilized by the trans-sulfuration pathway. Reductions in GSH and SAM as well as dysfunction in the trans-sulfuration pathway have been documented in the brains of Alzheimer's disease (AD) patients. Our previous *in vivo* study revealed that SAM administration attenuated oxidative stress induced by amyloid-β (Aβ) through the enhancement of GSH.
Objective: To investigate the effect of Aβ-induced oxidative stress on the trans-sulfuration pathway in astrocytes and neurons, respectively, and the protective effect of SAM on neurons.
Methods: APP/PS1 transgenic mice and the primary cultured astrocytes, neurons, and HT22 cells were used in the current study.
Results: SAM could rescue the low trans-sulfuration pathway activity induced by Aβ only in astrocytes, accompanying with increasing levels of Cys and GSH. The decrease of cellular viability of neurons caused by Aβ was greatly reversed when co-cultured with astrocytes with SAM intervention. Meanwhile, SAM improved cognitive performance in APP/PS1 mice.
Conclusion: In terms of astrocyte protection from oxidative stress, SAM might be a potent antioxidant in the therapy of AD patients.

Keywords: Amyloid-β, astrocytes, glutathione, S-adenosylmethionine, trans-sulfuration pathway

INTRODUCTION

Glutathione (GSH), the tripeptide containing thiol, is composed of glutamic acid, cysteine (Cys), and glycine. GSH is an important endogenous antioxidant that can protect cells from free radical damage [1]. Cys is the substrate limiting the production of GSH [2–4], therefore, the level of Cys is the key factor to maintain the balance of oxidation-antioxidation. Dietary methionine is activated by conversion to S-adenosylmethionine (SAM) in an ATP-dependent reaction catalyzed by methionine adenosyltransferase to yield S-adenosylhomocysteine (SAH), followed by homocysteine (HCY). HCY can be either re-methylated back to methionine using a methyl group provided by methyltetrahydrofolate or irreversibly converted into cystathionine by cystathionine β-synthase (CBS) via trans-sulfuration pathway [3]. Cys is generated from cystathionine by catalysis of cystathionine γ lyase (CSE). Thus, the

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trans-sulfuration pathway is thought to be the main source of Cys for GSH [4–6].

The trans-sulfuration pathway is particularly active in the liver [7] since about 50% of the Cys in GSH is derived from methionine through the trans-sulfuration pathway in the mammalian liver [8]. Low levels of GSH accompanied by damage of the trans-sulfuration pathway [9] have been found in many liver diseases, such as non-alcoholic fatty liver disease, alcoholic liver disease, drug-induced liver injury as well as hepatic fibrosis [10]. However, the very existence of this pathway in the brain has been once-controversial [5]. With the deepening of research, more and more evidence has confirmed that the existence of an intact trans-sulfuration pathway in the brain, and cell-specific differences in the trans-sulfuration pathway capacity [5]. Compared with the liver, the activity of the trans-sulfuration pathway in the brain is very weak, and the activities of the key enzyme CBS and CSE are 100 times lower than those in the liver [3]. As the most abundant subtype of glial cells in the central nervous system, astrocytes have a high trans-sulfuration pathway capacity [4] and more storage of GSH than those in neurons [11]. Extracellular GSH released from astrocytes is broken down into Cys, which is taken up by neurons and used to synthesize their own GSH [4, 12, 13].

Alzheimer's disease (AD) is a kind of neurodegenerative disorder characterized by cognitive impairment. The pathological features include the extracellular deposition of amyloid-β (Aβ) in senile plaques and intracellular formation of neurofibrillary tangles as well as neuronal death in the brain [14]. Aβ is a strong inducer of oxidative stress, which induces the generation of free radicals, cause oxidative stress damage and inflammatory reaction [15, 16]. Although the causes of AD are still unclear, oxidative injury is recognized as one of the important pathogenic mechanisms implicated in AD [17]. It is reported that a high level of HCY and reductions in GSH, SAM, and CBS protein in the AD brain, which make the brain more vulnerable to Aβ-induced toxicity [2, 18]. Moreover, disrupted Cys and GSH metabolism has been observed in AD [2]. Some studies outline the dysfunction in the trans-sulfuration pathway in the brains of both AD patients and AD animal models [19].

SAM is an important cell metabolite produced in the methionine cycle and is a direct methyl donor and the precursor of GSH. A few promising clinical studies with AD patients and extensive research of AD animal models exhibit the efficacy of SAM in improving cognitive performance [20, 21]. Our previous study with the Aβ-intrahippocampal injection rat model reveals that SAM administration attenuates oxidative stress induced by Aβ through enhancement of GSH and potentiates the antioxidant enzyme activities [22]. However, the efficacy of SAM only stays at brain tissue, it is unclear the effects of Aβ-induced oxidative stress on the trans-sulfuration pathway in astrocytes and neurons, respectively, and the intervention of SAM. In the present study, the APP/PS1 transgenic mice and the primary cultured astrocytes, neurons, and HT22 cells were used to reveal the astrocyte protection of neurons from Aβ-induced oxidative stress by SAM.

MATERIALS AND METHODS

Animals and treatment

Male APPswe/PS1dE9 (APP/PS1) mice and male wild-type C57BL/6J mice (C57) of 8-week-old (the ethical approval reference number were AEEI-2019-081, Beijing HFK Bioscience Co, Ltd. Beijing, China) were used in the present study. All animals were housed in the Experimental Animal Center of the Capital Medical University under conventional laboratory conditions (22–24°C 40–60% relative humidity) with ad libitum access to food and water and maintained on a standard 12/12 h light/dark-cycle. The mice were randomly divided into four groups (20 mice per group): C57 group, C57 + SAM group, APP/PS1group, and APP/PS1 + SAM group. SAM (6 mg/mL) was intragastricly administrated at 20 mg/kg weight every other day for seven months. The mice of C57 group and APP/PS1 group were administered with the same volume of saline. All experimental procedures complied with the Guidance for the Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of China.

Cell culture and treatment

Mouse hippocampal neuronal cell line (HT-22, undifferentiated) was obtained from Shanghai Langkang Biotechnology Co. Ltd. (China) and maintained at 37°C in a humidified atmosphere with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS) (Gibco).

Primary astrocyte culture

Thebrain was obtained from the postnatal 0-day-old Sprague-Dawley rat pups. After removing the cerebellum, blood vessels, and meninges, cerebral-cortices were isolated under a stereomicroscope. The cerebral cortices were then cut into small pieces, and digested with 0.25% trypsin for 5 min at 37°C. After being filtered through a 70 μm cell strainer (Biologix, China), the cell suspension was centrifuged, resuspended, and seeded in 60 mm dishes at a density of 1×10^6 cells/mL maintained in Dulbecco's modified Eagle's medium: Nutrient Mixture F-12 (Ham) (DMEM/F12) (Gibco) supplemented with 10% FBS (Gibco). After incubation for 1 h at 37°C, the cell suspension was collected and seeded in 25 cm^2 flasks maintained in DMEM/F12 (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin (Gibco, Invitrogen, USA). The medium was refreshed every 3 days. After 8–10 days, microglial cells were removed by shaking in an orbital shaker at 37°C with the shaking speed of 250 r/min for 18 h. The cells were transferred to 60 mm dishes and maintained in DMEM/F12 supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Cells from passage 3 were used for subsequent experiments. The purity of astrocytes was assessed by staining with the antibody of anti-GFAP (1 : 300, Cell Signaling Technology, Danvers, MA, USA, #3670).

Primary neuron culture

The cerebral cortices were isolated from postnatal 0-day-old Sprague-Dawley rat pups, then cut into small pieces, and digested with 0.125% trypsin for 15 min at 37°C. After being filtered through a 70 μm cell strainer (Biologix), the cell suspension was centrifuged, resuspended, and seeded onto poly-L-lysine (0.05 mg/ml)-coated 6-well plates at a density of 1 × 106 cells/mL maintained in DMEM/F12 (Gibco) supplemented with 2% B27 (Gibco) and 0.25% Glumax (Gibco). After 3 days, 2.5 μg/mL cytosine arabinoside (Sigma, USA) was added in the culture medium for 24 h to inhibit the proliferation of glial cells. Fifty percent of the medium was replaced every third day. The cells were maintained at 37°C with 5% CO2 for 14 days before subsequent experiments. The purity of neurons was assessed by staining with the antibody of anti-map-2 (1 : 300, Abcam, Cambridge, MA, USA, ab32454).

Cell co-culture

The transwell co-culture system was used. Astrocytes were cultured at a density of 5×10^5 on transwell inserts (pore size 0.4 μm, Corning, NY, USA) in 6-well plates, and the HT22 cells or primary cultured neurons were placed in 6-well plate.

Both Aβ_{1-42} and scrambled Aβ_{1-42} (peptide comprised of the same amino acid composition of Aβ_{1-42} but in a randomized sequence, China Peptides Co., Ltd, China) were aggregated by incubation in distilled saline at 37°C for 72 h before use. Aggregated Aβ_{1-42} (20 μM), aggregated scrambled Aβ_{1-42} (20 μM) were added into the medium co-incubated with or without SAM (100 μM in water, Yuanye Bio, China) for 72 h.

Cell viability assay

The cell viability was tested using Cell Counting Kit-8 (cck-8 kit) (Applygen, China) following the manufacturer's instruction. Astrocytes or HT22 cells were cultured in 96-well plates at a density of 5×10^4 cells/well. Primary neurons and co-culture cells were tested in 6-well plates. Before assay, the medium was replaced by fresh media and CCK-8 solution was added in each well and allowed to incubate for 1–1.5 h at 37°C. The absorbance was measured using microplate reader (Thermo Scientific, Waltham, MA, USA) at 450 nm. The absorbance in wells with only medium and CCK-8 solution was used as negative control. The percentage of cell viability was calculated as follows:

$$\text{Cell viability } (\%) = \left(\text{A450}\left[\text{sample}\right] - \text{A450}\left[NC\right]\right) / \left(\text{A450}\left[\text{control}\right] - \text{A450}\left[\text{NC}\right]\right) \times 100\%$$

Flow cytometric apoptosis analysis by annexin-PI staining

The cellular apoptosis was detected using annexin V-FITC apoptosis detection kit (Beyotime, China) following the manufacturer's instructions. Cells were harvested and carefully washed with PBS for three times. After centrifugation at 1000 g for 5 min, the cell pellets were resuspended in 195 μL annexin V binding buffer and mixed by adding 5 μL annexin V-FITC and 10 μL propidium iodide. The suspension was then incubated in the dark for 15 min at room temperature. The samples were analyzed by Guava easy cyte flow cytometer (Millipore, Darmstadt, Germany).

Western blot analysis

Cells or cerebral cortices of each group were harvested and homogenized in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mMNaCl, 1% NP-40, 0.1% SDS, and protease inhibitor cocktail (PPLYGEN, China). The extraction of membrane protein carried out using Membrane and Cytosol Protein Extraction Kit (Beyotime) following the manufacturer's instructions. Routine procedures were carried out as described previously [22]. The primary antibodies used in this study were as follows: rabbit anti-CBS polyclonal antibody (1 : 1000, Protein Tech, Chicago, IL USA, 14787-1-AP), rabbit anti-GCLC monoclonal antibody (1 : 5000, Abcam, ab190685), rabbit anti-xCT monoclonal antibody (1 : 5000, Abcam, ab175186), rabbit anti-ATP1A1 polyclonal antibody (1 : 1000,ProteinTech,14418-1-AP), rabbit anti-EAAT3 polyclonal antibody (1 : 1000, Protein Tech, 12686-1-AP), and mouse anti-GAPDH monoclonal antibody (1 : 10000, Protein Tech, 60004-1-lg). The quantification of the blots was carried out using Alpha Fluor Chem FC3system (Protein Simple, USA) and analyzed with Image J 16.0 (NIH, Bethesda, MD, USA). Each blot was quantified densitometrically, and then the data were obtained after being normalized to GAPDH blots. All original western blot images are shown in Supplementary Figure 1.

CBS activity assay

Cultured cells or cerebral cortices were harvested and 500 μl of CBS Assay Buffer was added to each sample, then homogenize on ice and centrifuge at 10,000 g, 4°C for 10 min. The supernatant was collected and detected by Cystathionine β Synthase Activity Assay Kit (Fluorometric) (BiovisionInc., Milpitas, CA, USA #K998-100) according to the manufacturer's instructions. The fluorescence intensity was measured using Twinkle LB 970 (Berthold, Bad Wildbad, Germany) with the excitation and emission wavelengths at 368 and 460 nm, respectively. All assays were performed in triplicate.

Quantification of SAM and HCY

Cultured cells or cerebral cortices were collected and homogenized in saline. After centrifugation at 1,500 g for 20 min at 4°C, the supernatant was collected for the quantitative analysis of SAM or HCY by enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone Corp, Wuhan, China) according to the manufacturer's protocols. The colorimetric reaction products were measured at 450 nm using microplate reader (Thermo Scientific). All assays were performed in triplicate.

Quantification of Cys

The production of Cys was measured by Cys ELISA kit (MeiBiao Biology Corp, Jiangsu, China) according to the manufacturer's instructions. The absorbance was measured using microplate reader (Thermo Scientific) at the wavelength of 450 nm. All assays were performed in triplicate.

Determination of GSH

Cultured cells or cerebral cortices of each group were harvested and homogenized. After centrifugation at 14,000 g for 10 min at 4°C, the supernatant was collected for the quantitative analysis of reduced GSH level according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The absorbance was measured using microplate reader (Thermo Scientific) at the wavelength of 405 nm. All assays were performed in triplicate.

Morris water maze test

The Morris water maze test was performed 7 months after SAM administration. A pool (diameter: 100 cm; height: 50 cm; depth of water: 30 cm) was filled with water (22 ± 1°C). A transparent platform (diameter: 8cm; height: 29 cm) was placed at the center of one quadrant in the pool, and the platform was approximately 1 cm beneath the water surface, invisible from the surface of the water. The water in the pool was dyed white. During the experiment, some specific graphics were fixed in someplace in the wall of the pool to provide additional cues for the mice to locate the platform. Before starting a hidden platform experiment, animals receive adaptive training. Briefly, during the acquisition trial (days 1–5), each mouse was trained to find a submerged platform within 60 s, and the latency, swimming speed and searching distance were recorded. If the mouse found the platform within 60 s, the time was recorded as the latency. Otherwise, the mouse was gently guided to the platform and allowed to remain on the platform for 10 s, and the latency was recorded as 60 s. For the probe trial (day 6), the platform was removed and the

mouse was allowed to swim freely for 60 s; the number of target platform crossings and the time spent and swimming distances in each quadrant were recorded [23].

Statistical analysis

The normality of all data was evaluated by Shapiro-Wilk test using SPSS software, version 21.0 (SPSS Inc., Chicago, USA). Data were analyzed by one-way ANOVA and two-way ANOVA followed by Tukey's *post hoc* test using Prism6 (GraphPad software, USA) software, and data were considered statistical significance as $p < 0.05$. All data were presented as mean ± standard deviation (SEM).

RESULTS

SAM rescued Aβ-induced low trans-sulfuration pathway activity in astrocytes, but not in HT22 cells

Firstly, APP/PS1 transgenic mice were used to get the knowledge to the levels of SAM and HCY under Aβ insult in the brain tissue. The level of SAM in the brain of APP/PS1 mice was reduced to 78.8% (Fig. 1A), while, HCY was increased to more than 2-fold, compared with C57 mice (Fig. 1D). After SAM administration for 7 months, the level of HCY was reduced to 69.6%, compared with APP/PS1 mice (Fig. 1D). Next, the effects of Aβ treatment on the levels of SAM and HCY were examined in either astrocytes or neurons, respectively. SAM levels were decreased to 70.0% and 72.4%, while, HCY levels were increased to 1.6-fold and 1.46-fold in the primary cultured astrocytes and HT22 cells under Aβ treatment, compared with the control group (Fig. 1B, C, E, F). The level of HCY decreased to 68.2% in the SAM intervention group in the primary cultured astrocytes (Fig. 1E), while, no significant changes were observed in HT22 cells with SAM intervention (Fig. 1F).

Since CBS is the key component of the trans-sulfuration pathway that catalyzes the conversion of HCY to cystathionine [8], CBS expression and activity were examined. There was no significant change in the expression of CBS protein in APP/PS1 transgenic mice (Fig. 2A), while the CBS activity was decreased to 54.3%, compared with C57 mice (Fig. 2D). SAM administration enhanced CBS activity to 1.71-fold,

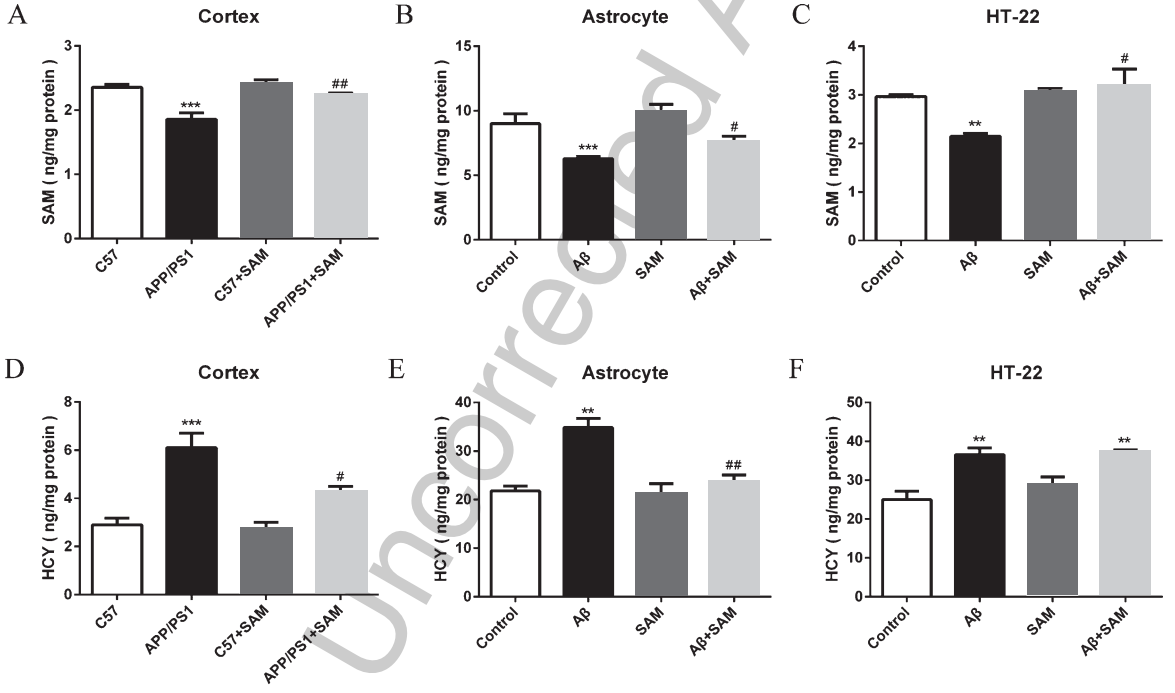

Fig. 1. SAM and HCY levels in APP/PS1 mice and cultured cells. The level of SAM and HCY were examined by ELISA in (A, D) cortex from APP/PS1 mice ($n = 5$) treated with or without SAM (20 mg/kg) every other day for seven months, (B, E) astrocytes ($n = 3$) and (C, F) HT22 cells ($n = 3$) treated with Aβ (20 μM) with or without SAM (100 μM) for 72 h. Data were expressed as mean ± SEM. $^{**}p < 0.01$; $^{***}p < 0.001$ versus C57 mice or control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus APP/PS1 mice or Aβ group. All *in vitro* experiments were performed independently 3 times and repeated 3 times.

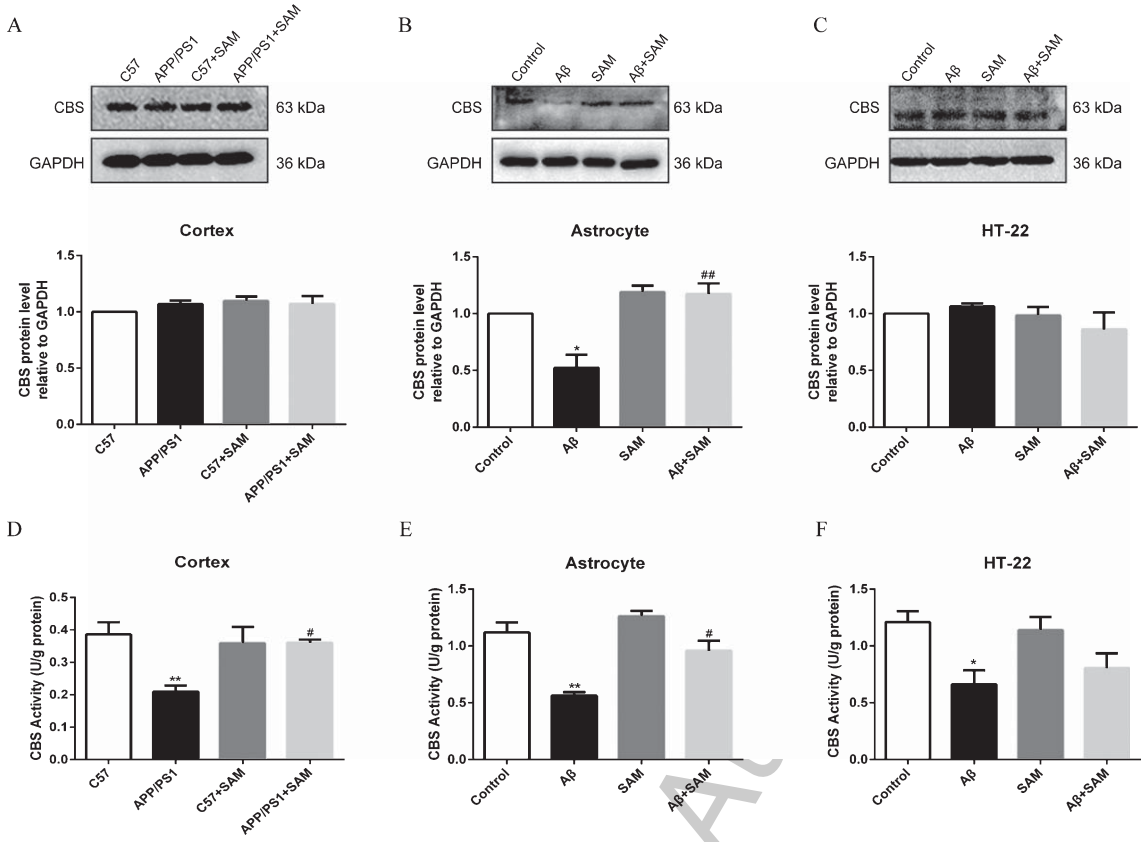

Fig. 2. CBS expression and activity in APP/PS1 mice and cultured cells. CBS protein levels were tested by western blot and CBS activity examined using CBS Activity Assay Kit in (A, D) cortex from APP/PS1 mice ($n = 5$–6) treated with or without SAM (20 mg/kg) every other day for seven months, (B, E) astrocytes ($n = 3$) and (C, F) HT22 cells ($n = 3$) treated with Aβ (20 μM) with or without SAM (100 μM) for 72 h. The expressions of the blots were densitometrically quantified, and the data were obtained after being normalized to GAPDH blots. Data were expressed as mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; versus C57 mice or control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus APP/PS1 mice or Aβ group. All *in vitro* experiments were performed independently 3 times and repeated 3 times.

compared with APP/PS1 mice (Fig. 2D).In astrocytes, the expression of CBS protein and CBS activity were decreased to 52.3% and 50.2% under Aβ treatment, respectively, and SAM intervention increased both protein level and activity of CBS (Fig. 2B, E). However, in HT22 cells, CBS activity was decreased to 54.8% without alteration in CBS protein expression under Aβ treatment, and SAM didnot restore CBS activity (Fig. 2C, F). These data suggested that the trans-sulfuration pathway was largely inhibited by Aβ, and SAM could rescue its activity only in astrocytes.

SAM increased low level of GSH induced by Aβ in astrocytes, but not in HT22 cells

Since GSH is the main product of the trans-sulfuration pathway [5], next the levels of GSH were measured. GSH level in the brain of APP/PS1 mice was decreased to 48.2%, compared with C57 mice, and SAM administration reversed it to 1.9-fold, compared with APP/PS1 transgenic mice (Fig. 3A). Similarly, the GSH level in Aβ treatment group was decreased to 72.1% and 43.2% of that in control group, and SAM intervention increased GSH levels to 1.3-fold and 1.47-fold, compared with Aβ treated group in astrocytes and in the astrocytes cultured medium, respectively (Fig. 3B, C). In HT22 cells, the GSH level in Aβ treatment group was decreased to 72.1% of that in control group, and SAM did not reverse it (Fig. 3D).

Cys is an important substrate required for GSH and is supplied through the trans-sulfuration pathway [2]. Cys level in APP/PS1 transgenic mice was decreased to 81.7%, and SAM restored it to nearly normal level, compared with C57 mice (Fig. 3E). In astrocytes and their cultured medium, the levels of Cys in Aβ treatment group were decreased to 46.6% and 63.1% of

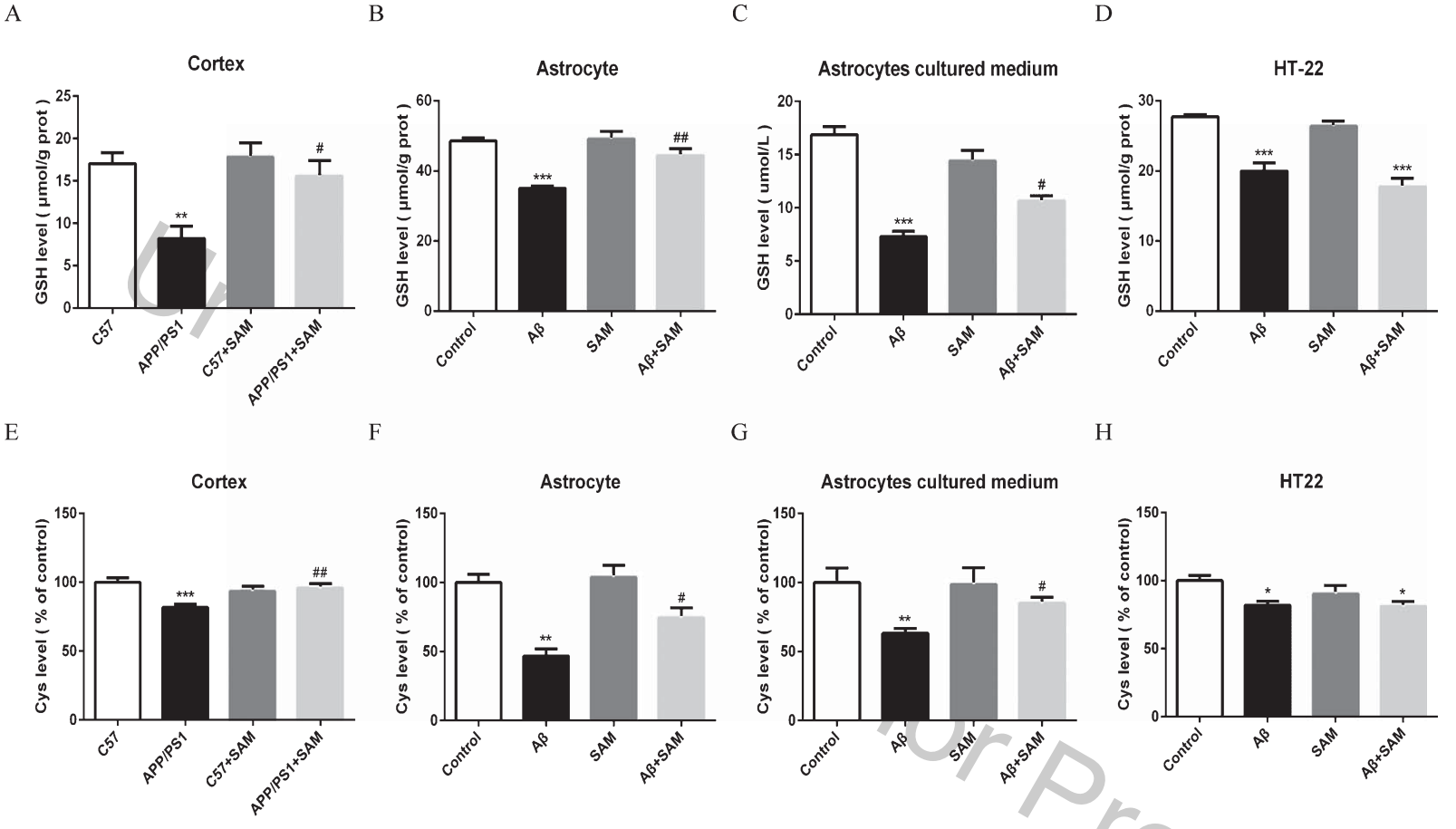

Fig. 3. GSH and Cys levels in APP/PS1 mice and cultured cells.The level of GSH tested by GSH assay kit and Cys were examined by ELISA respectively in (A, E) cortex from APP/PS1 mice ($n=5$) treated with or without SAM (20 mg/kg) every other day for seven months, (B, F) astrocytes ($n=3$), (C, G) astrocytes cultured medium ($n=3$), and (D, H) HT22 cells ($n=3$) treated with Aβ (20 μM) with or without SAM (100 μM) for 72 h. Data were expressed as mean ± SEM. *$p<0.05$; **$p<0.01$; ***$p<0.001$ versus C57 mice or control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ versus APP/PS1 mice or Aβ group. All *in vitro* experiments were performed independently 3 times and repeated 3 times.

that in the control group, while SAM intervention increased Cys level to 1.6-fold and 1.4-fold, compared with Aβ treatment group, respectively (Fig. 3F, G). In HT22 cells, the Cys level in Aβ treatment group was decreased to 81.8% of that in the control group, while SAM didnot reverse it (Fig. 3H).

Besides the trans-sulfuration pathway, Cys is also provided by cystine/glutamate exchange transporter (system x_c^-) for the production of GSH [24]. In astrocytes, the xCT (the functional subunit of system xc–) protein in Aβ treated group was significantly reduced to 33.5% compared with the control group; however, no effect of SAM on xCT expression (Fig. 4B). In addition, no changes of xCT expression were observed in APP/PS1 transgenic mice and HT22 cells (Fig. 4A, C). Most neuronal Cys uptake is mediated by EAAT3 [25]. The expression of EAAT3 protein was examined in HT22 cells to find that EAAT3 was decreased to 48.6% under Aβ treatment, and SAM intervention increased it to 2.7-fold, compared with Aβ treated group (Fig. 4D). In order to detect the effect of SAM on EAAT3 binding activity, the cells were incubated with 50 μM Cys at 37°C for 5 min before harvest and then detected the intracellular content of Cys. As shown in Fig. 4H, SAM could increase the content of intracellular Cys to 1.42-fold, compared with Aβ treatment group. These data implied that SAM could increase EAAT3 expression and activity in HT22 cells, which is conducive to neurons to uptake extracellular Cys for GSH synthesis.

GSH is synthesized by the consecutive action of the enzymes glutamate-cysteine ligase (GCL) and glutathione synthetase. The GCLC (the glutamate-cysteine ligase catalytic subunit) is the rate-limiting enzyme in GSH synthesis [26]. We found that GCLC protein in APP/PS1 transgenic mice was decreased to 75.1%, and SAM restored it to normal level, compared with C57 mice (Fig. 4E). However, there were no changes in astrocytes and HT22 cells (Fig. 4F, G). These data suggested that the synthesis of GSH is more sensitive to the substrate of Cys from the trans-sulfuration pathway in astrocytes.

SAM protected HT22 cells against Aβ injury when co-cultured with astrocytes

SAM did not reverse Aβ-induced low GSH level in HT22 cells; however, GSH level was increased by nearly 30% when supplementing of exogenous Cys (N-Acetyl-L-cysteine) (Fig. 5A). The *in vitro* study of astrocyte indicated that SAM could increase both GSH and Cys in astrocytes cultured medium (Fig. 3C, G), meanwhile, SAM increased EAAT3 expression and activity in HT22 cells (Fig. 4D, H). Next, the astrocytes-neuron co-culture system was used to explore the interplay of these two kinds of cells. When HT22 cells were co-cultured with astrocytes, the level of Cys in HT22 cells and the HT22 cultured medium were decreased to 80.1% and 70.0% of that in the control group when under Aβ treatment, while SAM increased Cys to 1.14-fold and 1.2-fold, compared with Aβ treatment group, respectively (Fig. 5B, C). Consistently, the GSH level in HT22 cells and co-cultured medium were increased to nearly 1.5-fold, compared with Aβ treatment group (Fig. 5D, E). Then, the cellular apoptosis and viability of HT22 cells in this co-culture system were tested. SAM could largely decrease the number of apoptotic cells by 44.2% and increased the cellular viability by 50.1%, compared with the HT22 cells cultured alone (Fig. 5F, G). Furthermore, the cellular viability of primary cultured neurons was also increased when co-cultured with astrocytes combined with SAM treatment (Fig. 5H). These results suggested that SAM could protect neurons from Aβ damage with the help of astrocytes.

SAM improved cognitive performance in APP/PS1 mice

Morris water maze was used to evaluate the spatial memory of the mice. APP/PS1 mice exhibited cognitive deficiency with prolonged escape latency and swimming distance, compared with C57 mice. The mice with SAM administration for 7 months demonstrated significant decreases in escape latency and swimming distance when compared with APP/PS1 mice (Fig. 6A, B). Moreover, longer times spent in the target quadrant and more times crossing the platform were observed in the mice with SAM administration compared with APP/PS1 mice (Fig. 6D, E). Swimming speed was not different among all the groups (Fig. 6C, F). These results implied that SAM might improve the learning and memory ability of APP/PS1 mice through protection of neurons against Aβ injury.

DISCUSSION

GSH assists in maintaining oxidative homeostasis by removing reactive oxygen species, reactive nitrogen species, and peroxides. GSH, hydrophilic compounds, can passively diffuse through the lipid border of the blood-brain barrier. It seems unlikely that direct transport of GSH would contribute consid-

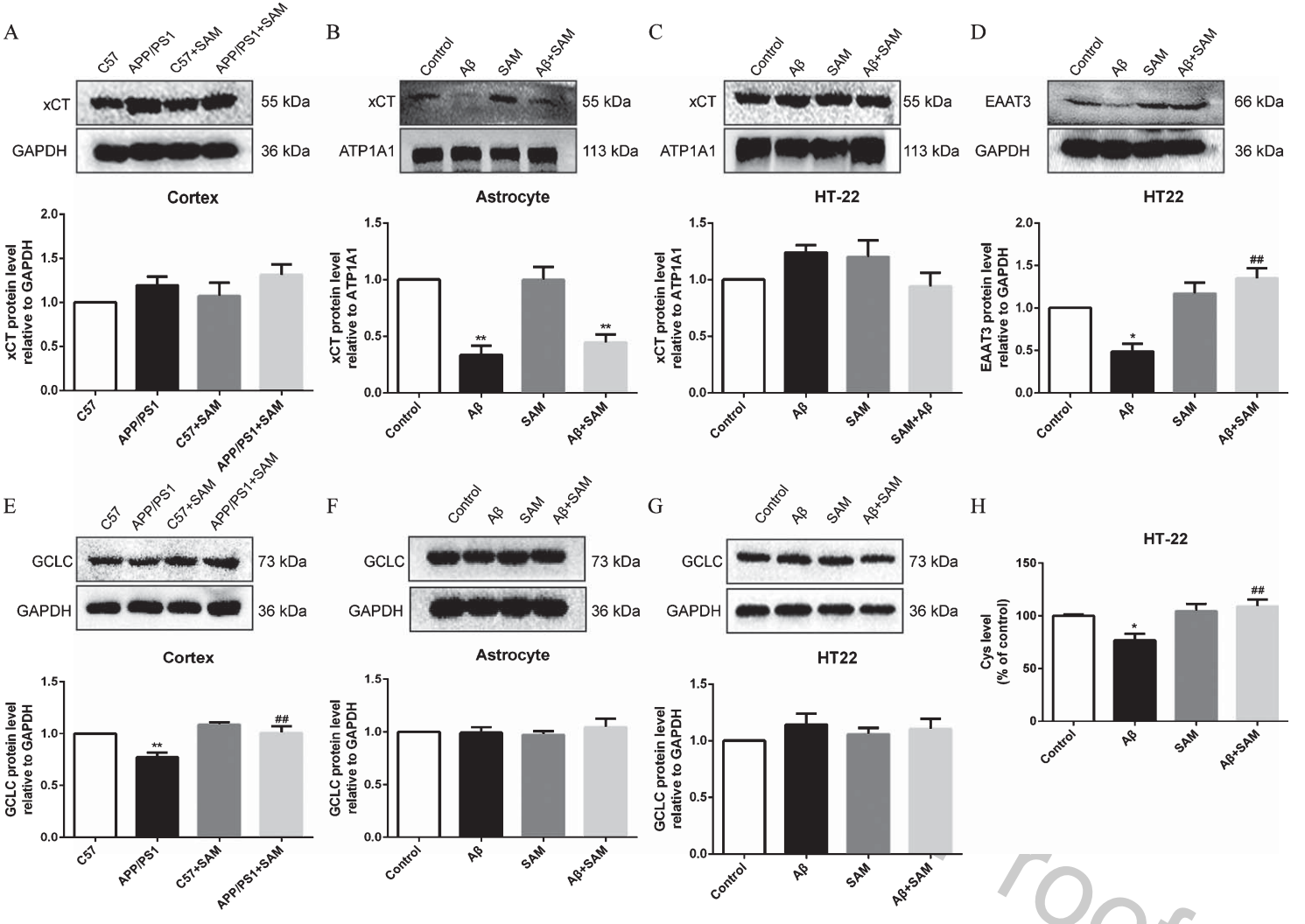

Fig. 4. The effect of SAM on xCT, EAAT3, and GCLC proteins under Aβ treatment. xCT and GCLC expressions were tested by western blot in (A, E) cortex from APP/PS1 mice ($n = 5$–6) treated with or without SAM (20mg/kg) every other day for seven months, (B, F) astrocytes ($n = 3$) and (C, G) HT22 cells ($n = 3$) treated with Aβ (20 μM) with or without SAM (100 μM) for 72 h. D) EAAT3 expressions were tested by western blot and (H) Cys level tested by ELISA in HT22 cells ($n = 3$) treated with Aβ (20 μM) with or without SAM (100 μM) for 72 h. The expressions of the blots were densitometrically quantified, and the data were obtained after being normalized to GAPDH blots. Data were expressed as mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$versus C57 mice or control group; $^{\#\#}p < 0.01$ versus APP/PS1 mice or Aβ group. All *in vitro* experiments were performed independently 3 times and repeated 3 times.

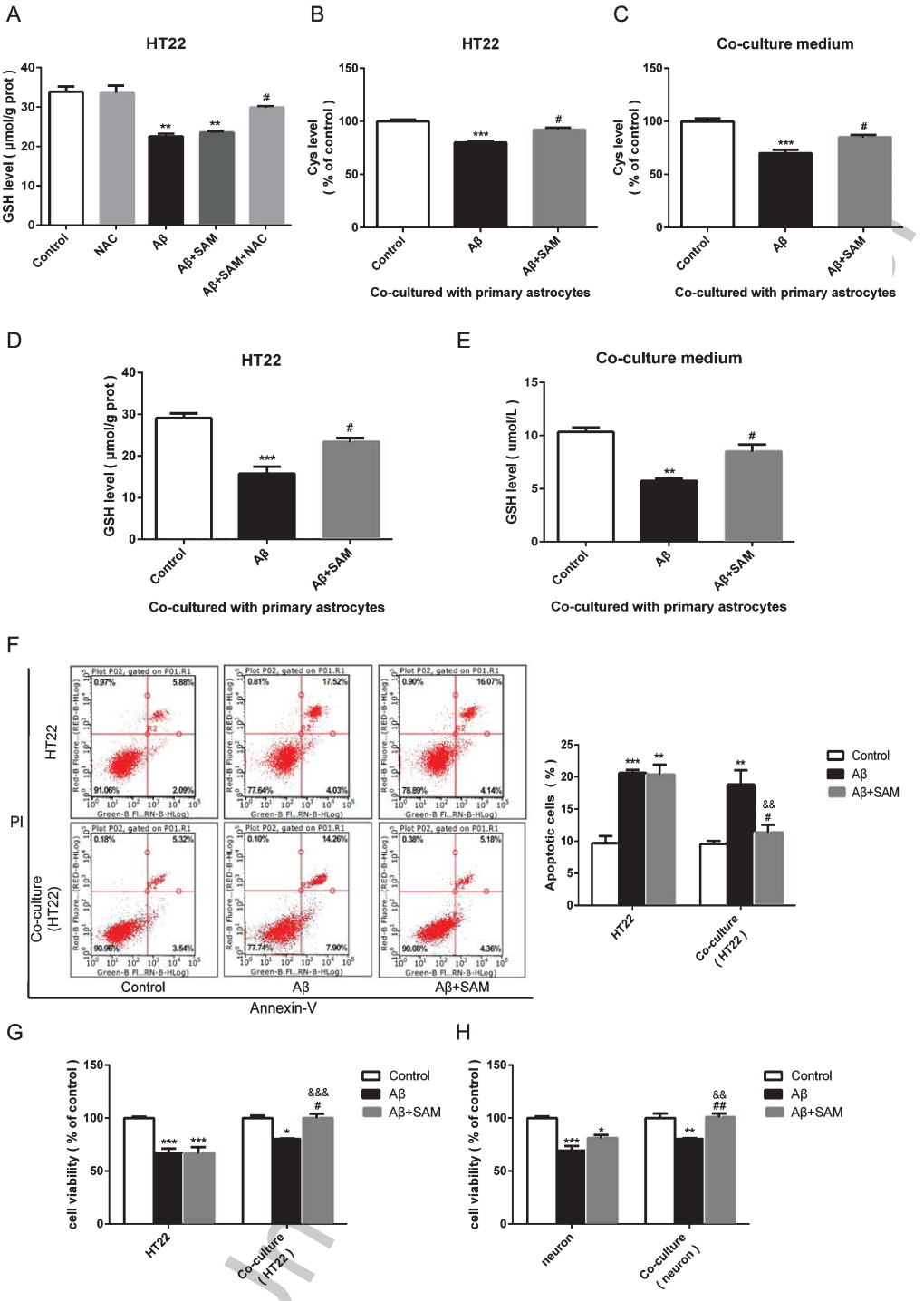

Fig. 5. SAM protected HT22 cells against Aβ injury when co-cultured with astrocytes. The levels of (A) GSH were examined in HT22 cells ($n = 3$) when treated with Aβ (20 μM) with or without SAM (100 μM), NAC(N-Acetyl-L-cysteine) (1 mM) for 72 h. The levels of Cys and GSH were examined in (B, D) HT22 cells ($n = 3$) and (C, E) the cultured medium ($n = 3$) when co-cultured with astrocytes under the treatment of Aβ (20 μM) with or without SAM (100 μM) for 72 h. F) Flowcytometric analysis for the determination of apoptosis tested by Annexin V-FITC/PI. Cellular viability tested by CCK-8 in (G) HT22 cells and (H) primary cultured neurons when single cultured ($n = 3$) or co-cultured with astrocytes ($n = 3$) treated with Aβ (20 μM) with or without SAM (100 μM) for 72 h. Data were expressed as mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ versus control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus Aβ group; $^{\&\&}p < 0.01$; $^{\&\&\&}p < 0.001$versus SAM intervention group single cultured. All *in vitro* experiments were performed independently 3 times and repeated 3 times.

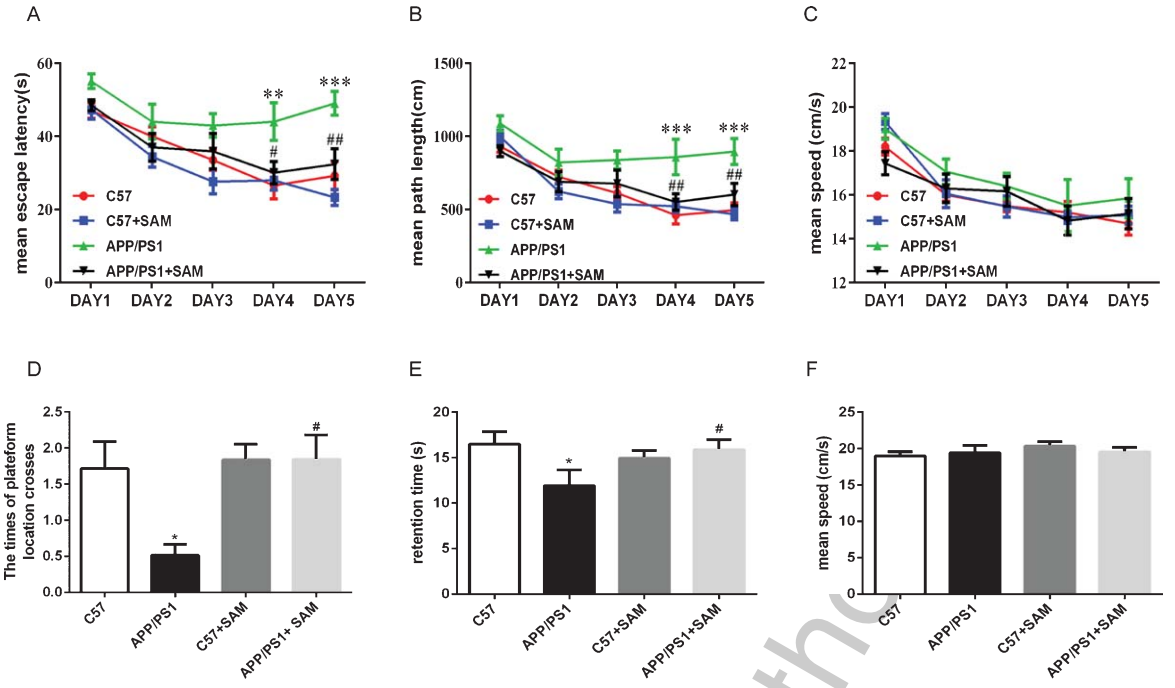

Fig. 6. Cognitive performance tested by Morris water maze in APP/ PS1 mice. The mice were subjected to Morris water maze test on a designed time for five consecutive days, (A) average escape latency, (B) average swimming distance, (C) mean speed in Positioning cruise experiment, (D) platform crossing time, and (E) time in target quadrant were measured ($n = 9$–15). The (F) mean swimming speed was calculated ($n = 9$–15). Data were expressed as mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ versus C57 mice; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus APP/PS1 mice.

erably to brain GSH content since most of the studies report that the cerebrospinal fluid concentration of GSH is 250–500 × lower than intracellular GSH levels in brain [27, 28]. Thus, intracellular synthesis is necessary for maintaining the large concentration difference. Cys is the substrate limiting the production of GSH and the immediate precursor of hydrogen sulfide and taurine, both of which have significant antioxidant properties [29, 30]. Cys must be supplied either from the extracellular medium imported cystine, its oxidized form, or *in situ* synthesis from methionine via the trans-sulfuration pathway. Astrocytes and microglial cells are the main sites of GSH production in the brain and import cystine, which is immediately reduced to Cys on entering the cytosol. In astrocytes, uptake of cystine provides approximately two-thirds of the Cys required for GSH synthesis. The remaining one third is derived from methionine via the trans-sulfuration pathway, but this contribution increases during oxidative stress [31].

The trans-sulfuration pathway has cell-specific differences and acts in a reserve capacity in astrocytes to boost GSH production when needed [32]. It is increasingly recognized that methionine is a significant source of Cys for GSH in astrocytes [3, 5, 30]. Methionine is converted to Cys through the methionine cycle and trans-sulfuration pathway. Flux through the pathway is increased in response to oxidative stress, meaning that a greater percentage of Cys for GSH originates from methionine rather than the extracellular compartment [3, 30]. It is believed that CBS is more important than CTH in astrocytes [33] and is primarily expressed in astrocytes [34]. An inherited deficiency in CBS results in a pathological accumulation of HCY which can cause oxidative stress due to a limited supply of Cys for GSH in the brain [35]. In our study, we found that SAM intervention could repair the damage of the trans-sulfuration pathway induced by Aβ to increase GSH level in astrocytes and APP/PS1 mice, especially in improving CBS activity. The protective effect of SAM was not seen in HT22 cells, which might be due to the weak ability of trans-sulfuration pathway in neurons. Besides, the enzyme of CBS can generate hydrogen sulfide (H_2S) using Cys or HCY as a substrate [32]. H_2S has several physiological and pathological actions in the brain, including the ability to protect neurons from oxidative stress and glutamate-

mediated excitotoxicity [36, 37]. In the present study, we do not focus on H_2S, and it deserves further study in this system in the future.

Uptake of cystine is also mediated by the x_c^- exchanger (*SLC7A11* carrier) that releases glutamate in a 1 : 1 ratio with cystine uptake. Basal xCT is expressed both in neurons and astrocytes of the cerebral cortex [38–40]. Some study finds that impaired GSH metabolism correlates with downregulation of xCT [41] and targeted deletion of the mice xCT gene lead to higher cystine/Cys ratios in blood plasma, elevated redox stress, and oxidative damage [42]. In addition, xCT over expression alone in astrocytes is sufficient to increase GSH synthesis/release and protect neurons against oxidative stress [24]. However, the system x_c^- can release glutamate which increases glutamate in extracellular to cause neurotoxicity [43]. Other research finds that microglia enhances the toxicity of Aβ by releasing glutamate through the system x_c^- [44] which seems to be a double-edged sword. Consistent with the above reports, our data showed that xCT protein decreased significantly in astrocytes accompanied by low-level GSH induced by Aβ, but not in HT22 cells. The reason for this inconsistency may be that the x_c^- is not the main channel protein to maintain the level of Cys in neurons. Meanwhile, the decrease of xCT protein was not found in APP/PS1 transgenic mice. Some research report that astrocytes in isolation are dependent upon the x_c^- exchanger for Cys to generate GSH, whereas the relationship is less critical *in vivo* [45]. In addition, alternative pathways or compensatory mechanisms operate to maintain an adequate supply of intracellular Cys and GSH when the animals in the absence of a functional x_c^- exchanger [46].

Moreover, Cys can be taken up by EAAT3 coded by SLC1A1/Slc1a1 [2]. Excitatory amino acid transporters (EAATs) are high-affinity Na+-dependent carriers of major importance in maintaining glutamate homeostasis in the central nervous system. Some research reports that the EAAT3 is responsible for about 90% of total Cys uptake in neurons [47] and believed to be important for the synthesis of intracellular GSH and subsequent protection from oxidative stress [48]. The mice knocked-out forEAAT3, which presents a decreased GSH content in neurons and early onset brain aging, exhibits significant biochemical and functional improvements when treated with the antioxidant *N*-acetyl-cysteine [49–51]. The reports about EAAT3 expressions in mouse models of AD are conflicting. Schaller et al. [52] report an increase in EAAT3 expression in AβPP23 mice, while a decrease in the expression of EAAT3 has been reported by Cassano et al. [53] in triple transgenic (3×Tg) AD mice. In humans, aberrant intracellular accumulation of a detergent-insoluble EAAT3 has been described in hippocampal neurons of AD patients, although the overall cortical expression of the transporter is comparable to controls [54]. Impairment of the PI3k/Akt pathway which can increase the translocation of EAAT3 to the cell surface has been demonstrated in the postmortem brains of AD patients, associated with insulin-like growth factor-1 (IGF-1) and insulin resistance [19]. Our results showed that the expression of EAAT3 protein was decreased under Aβ insult and SAM can improve either its expression or activity in HT22 cells. The underlying mechanisms need to be further explored.

The regulation of GSH synthesis occurs at the level of γ-glutamate-cysteine ligase (GCL) and GCLC is the rate-limiting enzyme in GSH synthesis [55]. Our results showed that GCLC expression was decreased in Aβ-induced and SAM could recover it in APP/PS1 mice. However, this change was not observed in the *in vitro* study, which may be due to the different systems and differences in the experimental processing. However, it is puzzling that the gene coding for this enzyme of GCLC and xCT is a recognized target of the antioxidant response transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) [56], but their changes are inconsistent in our model, which suggested that there may be other regulatory pathways [46].

SAM is an important molecule in normal cell function and survival. SAM is utilized by three key metabolic pathways: transmethylation, trans-sulfuration, and polyamine synthesis. In trans-sulfuration, SAM is converted via a series of enzymatic steps to Cys, a precursor of GSH which is a major cellular anti-oxidant. SAM has been used extensively to manage liver diseases of different etiologies in both humans and animals [57–59]. The effect of SAM has been attributed to its role in the enhancement of GSH stores in hepatocytes and decrease the production of the reactive oxygen species [34, 60–62]. SAM decreases HCY remethylation to methionine by allosterically inhibiting methyltetrahydrofolate reductase (MTHFR) and activates CBS activity [63] to stimulate reduced GSH synthesis in liver disease [64]. Moreover, SAM administration abolishes hypomethylation, diminishes the amyloid pathology and restores cognitive capabilities [65]. SAM reduces amyloid production,

increases spatial memory in TgCRND8 mice, and inhibits tau phosphorylation in TgCRND8 mice [21, 66, 67]. Dietary supplementation with SAM delays Aβ and tau pathology in 3×Tg-AD mice [68]. Furthermore, the administration of SAM could inhibit oxidative and inflammatory injury in AD animal models [69, 70].

Collectively, SAM can protect neurons against Aβ-induced injury by enhancing the trans-sulfuration pathway in astrocytes. This study will help to understand the protective mechanisms of SAM in brain, especially for AD patients.

ACKNOWLEDGMENTS

This research was supported by National Natural Science Foundation of China (No. 81571281 and No. 81870827).

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-0103r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-200103.

REFERENCES

[1] Steele ML, Ooi L, Münch G (2012) Development of a high-performance liquid chromatography method for the simultaneous quantitation of glutathione and related thiols. *Anal Biochem* **429**, 45-52.

[2] Paul BD, Sbodio JI, Snyder SH (2018) Cysteine metabolism in neuronal redox homeostasis. *Trends Pharmacol Sci* **39**, 513-524.

[3] McBean GJ (2012) The transsulfuration pathway: A source of cysteine for glutathione in astrocytes. *Amino Acids* **42**, 199-205.

[4] McBean GJ (2017) Cysteine, glutathione, and thiol redox balance in astrocytes. *Antioxidants (Basel)* **6**, 62.

[5] Vitvitsky V, Thomas M, Ghorpade A, Gendelman HE, Banerjee R (2006) A functional transsulfuration pathway in the brain links to glutathione homeostasis. *J BiolChem* **281**, 35785-35793.

[6] Chua S, Wu CJ, Chang HW, Hang CL, Chen CJ, Yang CH, Yip HK (2005) Impact of elevated plasma total homocysteine concentration on coronary atherosclerosis in Chinese patients with acute myocardial infarction undergoing primary coronary intervention. *Int Heart J* **46**, 181-193.

[7] Jung YS (2015) Metabolism of sulfur-containing amino acids in the liver: A link between hepatic injury and recovery. *Biol Pharm Bull* **38**, 971-974.

[8] Banerjee R, Zou CG (2005) Redox regulation and reaction mechanism of human cystathionine-beta-synthase: A PLP-dependent hemesensor protein. *Arch BiochemBiophys* **433**, 144-156.

[9] Chen Y, Han M, Matsumoto A, Wang Y, Thompson DC, Vasiliou V (2018) Glutathione and transsulfuration in alcohol-associated tissue injury and carcinogenesis. *Adv Exp Med Biol* **1032**, 37-53.

[10] Anstee QM, Day CP (2012) S-adenosylmethionine (SAMe) therapy in liver disease: A review of current evidence and clinical utility. *J Hepatol* **57**, 1097-1109.

[11] Dringen R (2000) Metabolism and functions of glutathione in brain. *Prog Neurobiol* **62**, 649-671.

[12] Steele ML, Fuller S, Patel M, Kersaitis C, Ooi L, Münch G (2013) Effect of Nrf2 activators on release of glutathione, cysteinylglycine and homocysteine by human U373 astroglial cells. *Redox Biol* **1**, 441-445.

[13] Dringen R, Brandmann M, Hohnholt MC, Blumrich EM (2015) Glutathione-dependent detoxification processes in astrocytes. *Neurochem Res* **40**, 2570-2582.

[14] Parihar MS, Brewer GJ (2010) Amyloid-β as a modulator of synaptic plasticity. *J Alzheimers Dis* **22**, 741-763.

[15] Murray IV, Liu L, Komatsu H, Uryu K, Xiao G, Lawson JA, Axelsen PH (2007) Membrane-mediated amyloidogenesis and the promotion of oxidative lipid damage by amyloid beta proteins. *J BiolChem* **282**, 9335-9345.

[16] Amin FU, Shah SA, Kim MO (2017) Vanillic acid attenuates Aβ1-42-induced oxidative stress and cognitive impairment in mice. *Sci Rep* **7**, 40753.

[17] Tönnies E, Trushina E (2017) Oxidative stress, synaptic dysfunction, and Alzheimer's disease. *J Alzheimers Dis* **57**, 1105-1121.

[18] Linnebank M, Popp J, Smulders Y, Smith D, Semmler A, Farkas M, Kulic L, Cvetanovska G, Blom H, Stoffel-Wagner B, Kölsch H, Weller M, Jessen F (2010) S-adenosylmethionine is decreased in the cerebrospinal fluid of patients with Alzheimer's disease. *Neurodegener Dis* **7**, 373-378.

[19] Hodgson N, Trivedi M, Muratore C, Li S, Deth R (2013) Soluble oligomers of amyloid-β cause changes in redox state, DNA methylation, and gene transcription by inhibiting EAAT3 mediated cysteine uptake. *J Alzheimers Dis* **36**, 197-209.

[20] Montgomery SE, Sepehry AA, Wangsgaard JD, Koenig JE (2014) The effect of S-adenosylmethionine on cognitive performance in mice: An animal model meta-analysis. *PLoS One* **9**, e107756.

[21] Fuso A, Nicolia V, Ricceri L, Cavallaro RA, Isopi E, Mangia F, Fiorenza MT, Scarpa S (2012) S-adenosylmethionine reduces the progress of the Alzheimer-like features induced by B-vitamin deficiency in mice. *Neurobiol Aging* **33**, 1482.e1-16.

[22] Li Q, Cui J, Fang C, Liu M, Min G, Li L (2017) S-adenosylmethionine attenuates oxidative stress and neuroinflammation induced by amyloid-β through modulation of glutathione metabolism. *J Alzheimers Dis* **58**, 549-558.

[23] Janus C (2004) Search strategies used by APP transgenic mice during navigation in the Morris water maze. *Learn Mem* **11**, 337-346.

[24] Shih AY, Erb H, Sun X, Toda S, Kalivas PW, Murphy TH (2006) Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. *J Neurosci* **26**, 10514-10523.

[25] Wang YL, Chen M, Huo TG, Zhang YH, Fang Y, Feng C, Wang SY, Jiang H (2017) Effects of glycyrrhetinic acid on GSH synthesis induced by realgar in the mouse hippocampus: Involvement of system [formula: see text], system [formula: see text], MRP-1, and Nrf2. *MolNeurobiol* **54**, 3102-3116.

[26] Valdovinos-Flores C, Gonsebatt ME (2012) The role of amino acid transporters in GSH synthesis in the blood-brain barrier and central nervous system. *NeurochemInt* **61**, 405-414.

[27] Johnson WM, Wilson-Delfosse AL, Mieyal JJ (2012) Dysregulation of glutathione homeostasis in neurodegenerative diseases. *Nutrients* **4**, 1399-1440.

[28] Kannan R, Kuhlenkamp JF, Jeandidier E, Trinh H, Ookhtens M, Kaplowitz N (1990) Evidence for carrier-mediated transport of glutathione across the blood-brain barrier in the rat. *J Clin Invest* **85**, 2009-2013.

[29] Qu K, Lee SW, Bian JS, Low CM, Wong PT (2008) Hydrogen sulfide: Neurochemistry and neurobiology. *NeurochemInt* **52**, 155-165.

[30] Vitvitsky V, Garg SK, Banerjee R (2011) Taurine biosynthesis by neurons and astrocytes. *J BiolChem* **286**, 32002-32010.

[31] Diwakar L, Ravindranath V (2007) Inhibition of cystathionine-gamma-lyase leads to loss of glutathione and aggravation of mitochondrial dysfunction mediated by excitatory amino acid in the CNS. *NeurochemInt* **50**, 418-426.

[32] Kabil O, Vitvitsky V, Xie P, Banerjee R (2011) The quantitative significance of the transsulfuration enzymes for H2S production in murine tissues. *Antioxid Redox Signal* **15**, 363-372.

[33] Enokido Y, Suzuki E, Iwasawa K, Namekata K, Okazawa H, Kimura H (2005) Cystathionine beta-synthase, a key enzyme for homocysteine metabolism, is preferentially expressed in the radial glia/astrocyte lineage of developing mouse CNS. *FASEB J* **19**, 1854-1856.

[34] Miyamoto R, Otsuguro K, Yamaguchi S, Ito S (2015) Neuronal regulation of expression of hydrogen sulfide-producing enzyme cystathionine β-synthase in rat spinal cord astrocytes. *Neurosci Res* **97**, 52-59.

[35] Longoni A, Bellaver B, Bobermin LD, Santos CL, Nonose Y, Kolling J, Dos Santos TM, de Assis AM, Quincozes-Santos A, Wyse A (2018) Homocysteine induces glial reactivity in adult rat astrocyte cultures. *MolNeurobiol* **55**, 1966-1976.

[36] Kimura H (2013) Physiological role of hydrogen sulfide and polysulfide in the central nervous system. *NeurochemInt* **63**, 492-497.

[37] Wang JF, Li Y, Song JN, Pang HG (2014) Role of hydrogen sulfide in secondary neuronal injury. *NeurochemInt* **64**, 37-47.

[38] Pow DV (2001) Visualising the activity of the cystine-glutamate antiporter in glial cells using antibodies to aminoadipic acid, a selectively transported substrate. *Glia* **34**, 27-38.

[39] Melendez RI, Vuthiganon J, Kalivas PW (2005) Regulation of extracellular glutamate in the prefrontal cortex: Focus on the cystine glutamate exchanger and group I metabotropic glutamate receptors. *J PharmacolExpTher* **314**, 139-147.

[40] Burdo J, Dargusch R, Schubert D (2006) Distribution of the cystine/glutamate antiporter system xc- in the brain, kidney, and duodenum. *J HistochemCytochem* **54**, 549-557.

[41] Lewerenz J, Maher P (2009) Basal levels of eIF2alpha phosphorylation determine cellular antioxidant status by regulating ATF4 and xCT expression. *J BiolChem* **284**, 1106-1115.

[42] Sato H, Shiiya A, Kimata M, Maebara K, Tamba M, Sakakura Y, Makino N, Sugiyama F, Yagami K, Moriguchi T, Takahashi S, Bannai S (2005) Redox imbalance in cystine/glutamate transporter-deficient mice. *J BiolChem* **280**, 37423-37429.

[43] de Groot J, Sontheimer H (2011) Glutamate and the biology of gliomas. *Glia* **59**, 1181-1189.

[44] Qin S, Colin C, Hinners I, Gervais A, Cheret C, Mallat M (2006) System Xc- and apolipoprotein E expressed by microglia have opposite effects on the neurotoxicity of amyloid-beta peptide 1-40. *J Neurosci* **26**, 3345-3356.

[45] Banjac A, Perisic T, Sato H, Seiler A, Bannai S, Weiss N, Kölle P, Tschoep K, Issels RD, Daniel PT, Conrad M, Bornkamm GW (2008) The cystine/cysteine cycle: A redox cycle regulating susceptibility versus resistance to cell death. *Oncogene* **27**, 1618-1628.

[46] Seib TM, Patel SA, Bridges RJ (2011) Regulation of the system x(C)-cystine/glutamate exchanger by intracellular glutathione levels in rat astrocyte primary cultures. *Glia* **59**, 1387-1401.

[47] Aoyama K, Watabe M, Nakaki T (2012) Modulation of neuronal glutathione synthesis by EAAC1 and its interacting protein GTRAP3-18. *Amino Acids* **42**, 163-169.

[48] Himi T, Ikeda M, Yasuhara T, Nishida M, Morita I (2003) Role of neuronal glutamate transporter in the cysteine uptake and intracellular glutathione levels in cultured cortical neurons. *J Neural Transm (Vienna)* **110**, 1337-1348.

[49] Duerson K, Woltjer RL, Mookherjee P, Leverenz JB, Montine TJ, Bird TD, Pow DV, Rauen T, Cook DG (2009) Detergent-insoluble EAAC1/EAAT3 aberrantly accumulates in hippocampal neurons of Alzheimer's disease patients. *Brain Pathol* **19**, 267-278.

[50] Liu Y, Vidensky S, Ruggiero AM, Maier S, Sitte HH, Rothstein JD (2008) Reticulon RTN2B regulates trafficking and function of neuronal glutamate transporter EAAC1. *J BiolChem* **283**, 6561-6571.

[51] Cao L, Li L, Zuo Z (2012) N-acetylcysteine reverses existing cognitive impairment and increased oxidative stress in glutamate transporter type 3 deficient mice. *Neuroscience* **220**, 85-89.

[52] Schallier A, Smolders I, Van Dam D, Loyens E, De Deyn PP, Michotte A, Michotte Y, Massie A (2011) Region and age-specific changes in glutamate transport in the AβPP23 mouse model for Alzheimer's disease. *J Alzheimers Dis* **24**, 287-300.

[53] Cassano T, Serviddio G, Gaetani S, Romano A, Dipasquale P, Cianci S, Bellanti F, Laconca L, Romano AD, Padalino I, LaFerla FM, Nicoletti F, Cuomo V, Vendemiale G (2012) Glutamatergic alterations and mitochondrial impairment in a murine model of Alzheimer disease. *Neurobiol Aging* **33**, 1121.e1-12.

[54] Bianchi MG, Bardelli D, Chiu M, Bussolati O (2014) Changes in the expression of the glutamate transporter EAAT3/EAAC1 in health and disease. *Cell Mol Life Sci* **71**, 2001-2015.

[55] Makar TK, Nedergaard M, Preuss A, Gelbard AS, Perumal AS, Cooper AJ (1994) Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures of chick astrocytes and neurons: Evidence that astrocytes play an important role in antioxidative processes in the brain. *J Neurochem* **62**, 45-53.

[56] Parfenova H, Leffler CW, Basuroy S, Liu J, Fedinec AL (2012) Antioxidant roles of heme oxygenase, carbon monoxide, and bilirubin in cerebral circulation during seizures. *J Cereb Blood Flow Metab* **32**, 1024-1034.

[57] Mato JM, Alonso C, Noureddin M, Lu SC (2019) Biomarkers and subtypes of deranged lipid metabolism in

non-alcoholic fatty liver disease. *World J Gastroenterol* **25**, 3009-3020.

[58] Lozano-Rosas MG, Chávez E, Velasco-Loyden G, Domínguez-López M, Martínez-Pérez L, Chagoya De Sánchez V (2020) Diminished S-adenosylmethionine biosynthesis and its metabolism in a model of hepatocellular carcinoma is recuperated by an adenosine derivative. *Cancer BiolTher* **21**, 81-94.

[59] Lu SC, Mato JM (2008) S-Adenosylmethionine in cell growth, apoptosis and liver cancer. *J Gastroenterol Hepatol* **23 Suppl 1**, S73-S77.

[60] Tang Y, Chu H, Cao G, Du X, Min X, Wan C (2018) S-Adenosylmethionine attenuates bile duct early warm ischemia reperfusion injury after rat liver transplantation. *Mol Immunol* **95**, 83-90.

[61] Llacuna L, Marí M, Lluis JM, García-Ruiz C, Fernández-Checa JC, Morales A (2009) Reactive oxygen species mediate liver injury through parenchymal nuclear factor-kappaB inactivation in prolonged ischemia/reperfusion. *Am J Pathol* **174**, 1776-1785.

[62] Cederbaum AI (2010) Hepatoprotective effects of S-adenosyl-L-methionine against alcohol- and cytochrome P450 2E1-induced liver injury. *World J Gastroenterol* **16**, 1366-1376.

[63] Prudova A, Bauman Z, Braun A, Vitvitsky V, Lu SC, Banerjee R (2006) S-adenosylmethionine stabilizes cystathionine beta-synthase and modulates redox capacity. *Proc Natl Acad Sci U S A* **103**, 6489-6494.

[64] Pascale RM, Peitta G, Simile MM, Feo F (2019) Alterations of methionine metabolism as potential targets for the prevention and therapy of hepatocellular carcinoma. *Medicina (Kaunas)* **55**, 296.

[65] Do Carmo S, Hanzel CE, Jacobs ML, Machnes Z, Iulita MF, Yang J, Yu L, Ducatenzeiler A, Danik M, Breuillaud LS, Bennett DA, Szyf M, Cuello AC (2016) Rescue of early bace-1 and global DNA demethylation by S-Adenosylmethionine reduces amyloid pathology and improves cognition in an Alzheimer's model. *Sci Rep* **6**, 34051.

[66] Shea TB, Chan A (2008) S-adenosyl methionine: A natural therapeutic agent effective against multiple hallmarks and risk factors associated with Alzheimer's disease. *J Alzheimers Dis* **13**, 67-70.

[67] Persichilli S, Gervasoni J, Di Napoli A, Fuso A, Nicolia V, Giardina B, Scarpa S, Desiderio C, Cavallaro RA (2015) Plasma thiols levels in Alzheimer's disease mice under diet-induced hyperhomocysteinemia: Effect of S-adenosylmethionine and superoxide-dismutase supplementation. *J Alzheimers Dis* **44**, 1323-1331.

[68] Lee S, Lemere CA, Frost JL, Shea TB (2012) Dietary supplementation with S-adenosyl methionine delayed amyloid-β and tau pathology in 3xTg-AD mice. *J Alzheimers Dis* **28**, 423-431.

[69] Cuello AC, Hall H, Do Carmo S (2019) Experimental pharmacology in transgenic rodent models of Alzheimer's disease. *Front Pharmacol* **10**, 189.

[70] Cavallaro RA, Nicolia V, Fiorenza MT, Scarpa S, Fuso A (2017) S-adenosylmethionine and superoxide dismutase 1 synergistically counteract Alzheimer's disease features progression in TgCRND8 mice. *Antioxidants (Basel)* **6**, 76.