α-lipoic acid can greatly alleviate the toxic effect of AGES on SH-SY5Y cells

GUILFEN NIU¹, JIANFEI GUO¹, YAQIANG TIAN², KEXIANG ZHAO³, JIAN LI¹ and QIAN XIAO³

¹Department of Endocrinology, Liaocheng City People's Hospital, Liaocheng, Shandong 252000; ²Department of Endocrinology, Liaocheng Brain Hospital, Liaocheng, Shandong 252000; ³Department of the Elderly, Chongqing Medical University, Chongqing 400016, P.R. China

Received May 18, 2016; Accepted January 22, 2018

DOI: 10.3892/ijmm.2018.3477

Abstract. The aim of the study was to explore the influence of α-lipoic acid (α-LA) on the cytotoxicity of advanced glycation end-products (AGES) against SH-SY5Y cells. AGE-bovine serum albumin (BSA) was incubated in vitro using SH-SY5Y cells as a target model, and the control group was set. Cells were exposed to AGE-BSA, and α-LA was selectively added to the cells. Cell growth and death was determined by the MTT assay, which measures cellular metabolic rate, lactate dehydrogenase (LDH) leakage rate and cellular axonal length. Immunocytochemistry was employed to detect the expression of β-amyloid (Aβ) protein in cells, and mRNA expression of amyloid precursor protein (APP) and the receptor for AGE (RAGE) were assayed by RT-PCR. The metabolism of MTT was clearly increased, the rate of LDH leakage was significantly decreased, and axonal length was significantly increased in cells treated with α-LA (0.1 g/l) as compared to untreated cells. Furthermore, the expression levels of Aβ protein were also decreased. In addition, α-LA (0.1 g/l) markedly inhibited the expression of RAGE mRNA, and did not influence APP mRNA expression as compared the control group. α-LA (0.1 g/l) was effective at dampening the cytotoxicity of AGES against SH-SY5Y cells.

Introduction

It has been reported that diabetes may be an independent risk factor for dementia (1,2). The risks of cognitive impairment and Alzheimer's disease (AD) in type-2 diabetic patients were 1.5- and 1.6-fold of the normal population, respectively (3). Furthermore, the degree of brain atrophy seen in AD patients complicated by diabetes was more severe than that seen in AD patients, indicating that diabetes could accelerate the brain aging process of AD patients (4).

Advanced glycation end-products (AGES) are formed by the irreversible reaction between reducing sugar (glucose) and protein or lipid free radicals (5). The levels of AGES in diabetes patients were <5-fold that seen in non-diabetes individuals, which also plays a very important role in the occurrence and development of chronic diabetes-related complications (6). It was reported that AGES were present in the senile plaque (SP) and neurofibrillary tangles (NFTs) in the brain of AD patients (7). When AGE accumulation increased, it stimulated glial cells to generate inflammatory factors and increased RAGE expression, making the inflammatory reaction even more severe (8). However, AGES-RAGE could initiate distinct signaling pathways including p21/Ras, MAP kinase and NF-κB in an oxidative stress-dependent manner, and enhance neuronal expression of β-secretase. This process could further stimulate the generation of some cytokines, growth factors and adhesion molecules, and promote cell proliferation and vascular permeability. In addition, it also caused macrophage migration, stimulated endothelin formation, and promoted the synthesis of proteoglycans and fibers, which thus influenced β-amyloid (Aβ) protein secretion (9).

AD was first described by and named after Alzheimer in 1907, which was a primary degenerative disease that occurred in the elderly population. The typical pathologic changes included SP, NFTs, neuronal loss and axonal abnormalities, including astrocyte and microglial cell responses, and vascular amyloid changes. The major component of SP is Aβ, which is derived from the degradation of the amyloid precursor protein (APP) via the Aβ formation pathway. Thus, the influence of APP expression will indirectly regulate formation of Aβ protein.

Aβ is composed of 39-43 amino acids with a β-pleated sheet structure. Under normal physiological conditions, Aβ is soluble, its formation, degradation and elimination are in dynamic balance. In the brain of the AD patient, if such balance is destroyed, Aβ will form insoluble precipitation deposits by aggregation and might further lead to SP, which is the initiating factor for the occurrence of AD. Therefore, inhibition of Aβ formation plays a highly significant role in...
AD treatment. Thus, identifying an inhibitory drug therapy will also be very important in the treatment of AD.

α-lipoic acid (α-LA) is a natural product that was primarily extracted from pork liver by Reed in 1951. It is an indispensable substance in the tricarboxylic acid cycle and serves as a cofactor for pyruvate dehydrogenase. α-LA is metabolized to dihydrolipoic acid in vivo, and exists in the forms of LA and dihydrolipoic acid with synergistic effects that form a universal antioxidant. α-LA not only eliminates reactive oxygen and reduce oxidative stress levels, but it also increase GSH concentrations in tissues and prevents sharp decreases in GSH after oxidative damage of tissues (10).

It has been demonstrated that α-LA could improve the learning and memory functions in a rat model (11,12), and increased glucose metabolism and insulin sensitivity. Moreover, it could inhibit the developments of diabetic cardiovascular disease, diabetic cataract, diabetic peripheral nerve and autonomic neuropathy. Hager et al (13) attempted to use α-LA in the treatment of AD. In that study, 9 patients were orally administrated LA for 1 year, which was the first attempt in the LA-mediated treatment of AD in the clinic. It was found that the cognitive function of the patients was greatly improved by neuropsychological tests (13). However, the mechanism responsible for the ability of LA to protect neurons remains unclear.

In an in vitro study, Zhang et al found that LA could combat the damage that was mediated by the Aβ peptide and H2O2 on mouse neurons by activating the PKB/Akt signaling pathway (14). In the latest study, it was also found that α-LA inhibited Aβ protein aggregation, reduced Aβ fiber formation and depolymerized the aggregated Aβ (15). Nevertheless, studies of inhibiting Aβ formation remain very limited.

In the current study, we employed α-LA to treat cells, following which, we observed the protective effect on neurons and explored the possible mechanisms involved in an attempt to provide a theoretical basis for its application in the near future.

Materials and methods

The source of the cell-line and materials. SH-SY5Y cells were provided by The Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). D-glucose 1.1.2 (Amresco, LLC., Solon, OH, USA); MEM culture medium (HyClone, Logan, UT, USA); fetal bovine serum (Zhejiang Tianhang Biotechnology Co., Ltd., Hangzhou, China); 0.1% pancreatic enzymes, 0.02% EDTA, BSA and MTT (Beifangtongzheng); lactate dehydrogenase (LDH) test kit (Amresco, LLC., Solon, OH, USA); MEM culture medium provided by The Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China).

Preparation for AGE-BSA. FBS 5 g/l, D-glucose 0.5 M, penicillin 100 μ/l, streptomycin 100 μ/l and EDTA 1 mmol/l were dissolved in PBS (pH 7.4), then filtered by 0.22 μm microfiltration membrane and incubated at 37°C avoiding light for three months. The systems with the same FBS but under conditions of different glucose concentrations were used as control. PBS (pH 7.4) was used to dialyze the unbound glucose before use. The solution was stored at -20°C. AGEs was detected by a fluorospectrophotometer (4).

Grouping. First, the cells were divided into three groups randomly. Group 1 was the control group without any processing; group 2 was treated by different BSA concentrations (terminal concentrations of 0, 20, 40, 80, 160 and 320 μg/ml) for 48 h; and group 3 was treated by different AGE-BSA concentrations (terminal concentrations of 0, 20, 40, 80, 160 and 320 μg/ml) for 48 h. According to the results of the above experiment, group 4 was added, which represented cells treated by different α-LA concentrations (terminal concentrations of 0.01, 0.1 and 1 g/l) to intervene in cells 30 min prior to AGE-BSA (160 μg/ml) treatment, which was then added to treat the cells for 48 h. According to the result of group 4, group 5 was added, which included treatment by α-LA (0.1 g/l) to intervene cells 30 min prior to AGE-BSA (80 μg/ml) treatment for 48 h. The cell growth state were assayed by MTT metabolic rate, LDH leakage rate and cell axonal length. Immunocytochemistry was used to detect Aβ protein expression. Further, mRNA expression of APP and RAGE were tested by RT-PCR wherein the AGE-BSA concentration was chosen that slightly influenced cell growth. The concentration in each control group was found to be consistent with group 3.

MTT assay. SH-SY5Y cells were seeded at a density of 5x10^4/ml in 96 well-plates and incubated in 200 μl medium. Serum-free medium was added to replace the original medium after 24 h of culture. The cells were randomly divided into five groups. Group 1 was added with different BSA concentrations (0, 20, 40, 80, 160 and 320 μg/ml) for 48 h; group 2 was treated by different AGE-BSA concentrations (0, 20, 40, 80, 160 and 320 μg/ml) for 48 h. Group 3 was treated by different α-LA concentrations (0.01, 0.1 and 1 g/l) to intervene in cells 30 min earlier and then AGE-BSA...
(160 µg/ml) was added to treat cells for 48 h. Group 4 was added to introduce treatment of cells with α-LA (0.1 g/l) for 30 mins followed by treatment by AGE-BSA (80 µg/ml) for 48 h. Group 5 was a control treatment in the absence of any treatment. After 48 h of treatment, 5 mg/ml of MTT reagent (20 µl) was added to each well. The cells were further incubated for 4 h at 37˚C, which were then collected, centrifuged and washed once in culture medium without serum. DMSO (150 µl) was added to each well and shaken for 10 min to completely dissolve the formazan crystals. The optical density (OD) at a test wavelength of 570 nm was read by an automatic microplate reader. The cell viability in the control group was set as 100%, and the MTT metabolic rate was calculated by the ratio of OD to the control.

**LDH release assay.** SH-SY5Y cells were seeded at 5x10^4/ml in 24 well-plates and incubated in 1 ml medium. Medium without serum was added to replace the original after 24 h. The groups were the same as described for the MTT assay. Culture medium in each group was taken after 48 h, and OD values of the medium were measured by ultraviolet spectrophotometry at 440 nm according to the manufacturer's instruction. Furthermore, the best sample volume was obtained from the standard curve.

**Detection for cell axon length.** SH-SY5Y cells were seeded at a density of 5x10^4/ml in 35 mm culture flasks. Serum-free medium was added to replace the original after 24 h. The groups were the same as that described for the MTT assay. Photographic images of cells (random sampling of 30 cells) were taken 48 h after treatment by inverted phase contrast microscopy and the cell axon length was analyzed by pathologic image analysis.

**Detection of APP mRNA and AGER mRNA by RT-PCR.** The β-actin amplified fragment was 318 bp. Forward primer: 5'-ATCATGTTTGAGACCTTCAACA-3'. Reverse primer: 5'-CATCTCTTGATCGAAGTCCA-3'. The APP amplified fragment was 704 bp. Forward primer: 5'-CACATCCCTGGCTTTACGGTTC-3'. Reverse primer: 5'-TCAAGTGAGGGAGTTGC-3'. The RAGE: amplified fragment was 376 bp. Forward primer: 5'-GGCTGGTTATCAAGG-3'. Reverse primer: 5'-ACAGGTACGGTTACGGTTC-3'. Detection of SH-SY5Y intracellular Aβ levels by immunocytochemistry. Wells with SH-SY5Y cells were seeded at a density of 5x10^4/ml in 6-well plates. A small slide was placed in the plate before seeding. The assigned groups were as described for the MTT assay.

**Statistical analysis.** All data were analyzed by SPSS v.13.0 (SPSS, Inc. Chicago, IL, USA), and Microsoft Excel. The data are represented as mean ± SD. The comparisons of measured data among multiple groups were analyzed by one-way analysis of variance to determine the homogeneity test of variance. An α value of P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Fluorescence identification for AGEs-BSA.** After 90 days of incubation of cells with BSA and D-glucose, the fluorescence spectrum of AGEs-BSA showed that the excitation spectrum peak and emission spectrum peak were at 360 and 446 nm, respectively, in accordance with the characteristics of AGEs. However, that of the control BSA group had no such feature (Fig. 1).

![Figure 1. Expression of APP mRNA in SH-SY5Y cells dose-dependently treated by BSA and AGE-BSA. BSA, bovine serum albumin; AGE, advanced glycation end-product; APP, amyloid precursor protein.](image-url)
Table I. Concentration-dependent effect of BSA/AGE-BSA at 48 h on the MTT metabolic rate of SH-SY5Y cells.

<table>
<thead>
<tr>
<th>Groups (µg/ml)</th>
<th>BSA group</th>
<th>AGE-BSA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1±0</td>
<td>1±0</td>
</tr>
<tr>
<td>20</td>
<td>1.0019±0.0213</td>
<td>0.9902±0.0143</td>
</tr>
<tr>
<td>40</td>
<td>1.0001±0.0222</td>
<td>0.9346±0.0209&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>80</td>
<td>0.9952±0.0222</td>
<td>0.8783±0.0188&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>160</td>
<td>0.9922±0.0213</td>
<td>0.6434±0.0178&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>320</td>
<td>0.9928±0.0256</td>
<td>0.3406±0.0124&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD. vs. Control, <sup>a</sup>P<0.05; vs. BSA, <sup>b</sup>P<0.01. BSA, bovine serum albumin; AGE, advanced glycation end-product.

Table II. Concentration-dependent effect of BSA/AGE-BSA on LDH release by SH-SY5Y cells.

<table>
<thead>
<tr>
<th>Groups (µg/ml)</th>
<th>LDH leakage rate (µ/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>431.15±65.19</td>
</tr>
<tr>
<td>20</td>
<td>431.15±65.19</td>
</tr>
<tr>
<td>40</td>
<td>438.21±13.06</td>
</tr>
<tr>
<td>80</td>
<td>451.83±10.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>160</td>
<td>453.90±17.85</td>
</tr>
<tr>
<td>320</td>
<td>447.80±24.61</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD. vs. Control, <sup>a</sup>P<0.05; vs. control, <sup>b</sup>P<0.01. BSA, bovine serum albumin; LDH, lactate dehydrogenase; AGE, advanced glycation end-product; SD, standard deviation.

level at 320 µg/ml (P<0.01). A comparison of the AGE-BSA concentration group and the BSA alone group at the same concentration, demonstrated that the rate was significantly reduced at 20, 80, 160, and 320 µg/ml (P<0.05). Although the group treated with 20 µg/ml was shorter than the control group, it was not statistically significant (Table I).

Compared with the control group, there was no significant difference on LDH release in the BSA treated groups. AGE-BSA at a concentration of 40 µg/ml increased the release of LDH significantly (P<0.05) and reached the highest level at 320 µg/ml (P<0.01). The comparison of the AGE-BSA concentration group and the BSA alone group at the same concentrations demonstrated that the rate was significantly reduced at 40, 80, 160 and 320 µg/ml (P<0.05). Although the group treated with 20 µg/ml was lower than the control group, it was not statistically significant (Table II).

Compared with the control group, there was no significant difference in cell axon length in the BSA treated groups. In addition, 40 µg/ml AGE-BSA shortened the length significantly (P<0.05) and shortened gradually as concentration increased until reaching a peak at 320 µg/ml (P<0.01). The comparison of the AGE-BSA concentration group and the BSA alone group at the same concentrations demonstrated that the length was significantly short at 40, 80, 160 and 320 µg/ml (P<0.05). Although the group treated with 20 µg/ml was shorter than that of the control group, the observation was not significantly different (Table III).

Influence of BSA/AGE-BSA concentration on Aβ protein expression in SH-SY5Y cells. The color of positive Aβ protein expression was brownish red. In the control group, Aβ protein expression was decreased in the cytoplasm. As compared with control group, there was no significant change in terms of Aβ protein expression in the cytoplasm of cells that were dose-dependently treated by BSA. However, those treated by different AGE-BSA concentrations showed a significant difference (P<0.05). Compared with the corresponding BSA concentration group, Aβ protein expression in the cytoplasm was significantly increased following treatment with AGE-BSA (P<0.05; Table IV).

Influence of AGE-BSA concentrations on APP mRNA expression in SH-SY5Y cells. The group with the least influence on the state of cell growth was chosen. Compared with the control group, there was no significant difference on APP mRNA expression following treatment with AGE-BSA, or the BSA groups (Table V).

Influence of BSA/AGE-BSA concentration on RAGE mRNA expression in SH-SY5Y cells. Compared with control group, there was no significant difference on RAGE mRNA expression following treatment with BSA alone. In the AGE-BSA group, the RAGE mRNA expression was significantly increased at 20 µg/ml (P<0.05). Moreover, at 40 µg/ml and 80 µg/ml, RAGE mRNA expression showed a significant difference as compared with the control group (RAGE mRNA). As compared with the corresponding BSA concentration group, RAGE mRNA expression was significantly increased following treatment with AGE-BSA (P<0.05; Table VI and Fig. 2).
Observation of cell growth state following dose-dependent intervention with α-LA. According to the above results, the cells in the AGE-BSA (160 µg/ml) group were chosen. In the α-LA (0.1 g/l) intervention group, the MMT metabolic rate significantly increased, LDH release significantly decreased, and axonal length significantly increased as compared with that of the control group (P<0.05). Compared with the AGE-BSA (160 µg/ml) group, it was noted that the α-LA (0.01 g/l) intervention group showed an increased MMT metabolic rate, a decreased LDH release rate and increased axonal length as compared that of the control group - although this was not significantly different. It is interesting to note that the protective effect in the α-LA (1 g/l) intervention group disappeared. Compared with the control group, there was no evident changes in MTT metabolic rate or axonal length, and yet LDH release slightly increased (P>0.05; Table V). According to the above described results, it was found that α-LA (0.1 g/l) was protective for SH-SY5Y cells under treatment by 160 µg/ml AGE-BSA. Thus, we selected the cells in the AGE-BSA (80 µg/ml) group, and used α-LA at 0.1 g/l to intervene in the cells. Compared with the AGE-BSA (80 µg/ml) group, the Aβ expression significantly decreased in the intervention group (P<0.01; Table VII).

Table IV. Dose-dependent effect of different concentrations of BSA/AGE-BSA on expression of Aβ protein in SH-SY5Y cells.

<table>
<thead>
<tr>
<th>Groups (µg/ml)</th>
<th>BSA group</th>
<th>AGE-BSA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.129±0.025</td>
<td>0.129±0.025</td>
</tr>
<tr>
<td>20</td>
<td>0.162±0.017</td>
<td>0.167±0.029&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>0.125±0.022</td>
<td>0.226±0.076&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>80</td>
<td>0.128±0.018</td>
<td>0.309±0.073&lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table V. Dose-dependent effect of BSA/AGE-BSA on the expression of APP mRNA in SH-SY5Y cells.

<table>
<thead>
<tr>
<th>Groups (µg/ml)</th>
<th>APP mRNA/actin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA group</td>
<td>AGE-BSA group</td>
</tr>
<tr>
<td>0</td>
<td>0.284±0.033</td>
</tr>
<tr>
<td>20</td>
<td>0.290±0.032</td>
</tr>
<tr>
<td>40</td>
<td>0.289±0.027</td>
</tr>
<tr>
<td>80</td>
<td>0.288±0.019</td>
</tr>
</tbody>
</table>

Table VI. Dose-dependent effect of BSA/AGE-BSA on mRNA expression of RAGE in SH-SY5Y cells.

<table>
<thead>
<tr>
<th>Groups (µg/ml)</th>
<th>RAGE mRNA/actin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA group</td>
<td>AGE-BSA group</td>
</tr>
<tr>
<td>0</td>
<td>0.491±0.017</td>
</tr>
<tr>
<td>20</td>
<td>0.494±0.027</td>
</tr>
<tr>
<td>40</td>
<td>0.507±0.013</td>
</tr>
<tr>
<td>80</td>
<td>0.486±0.010</td>
</tr>
</tbody>
</table>

Table VII. Levels of MTT metabolic rate, LDH release rate and axonal length in SH-SY5Y cells following intervention by LA before treatment with 160 µg/ml AGE-BSA.

<table>
<thead>
<tr>
<th>Groups (g/l)</th>
<th>MTT metabolic rate (µl)</th>
<th>LDH leakage rate (µ/l)</th>
<th>Axon length (Pixel total points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.643±0.021</td>
<td>1608.22±141.37</td>
<td>21.13±5.61</td>
</tr>
<tr>
<td>0.01</td>
<td>0.684±0.022</td>
<td>1538.40±102.07</td>
<td>22.33±4.81</td>
</tr>
<tr>
<td>0.1</td>
<td>0.840±0.068&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1151.70±146.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.11±7.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.630±0.029</td>
<td>1662.52±110.82</td>
<td>20.89±3.68</td>
</tr>
</tbody>
</table>

Table VIII. Levels of Aβ protein expression in SH-SY5Y cells that were intervened by LA before being treated with 80 µg/ml AGE-BSA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aβ protein (OD value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 µg/ml AGE-BSA</td>
<td>0.309±0.073</td>
</tr>
<tr>
<td>0.1 g/l LA</td>
<td>0.213±0.046&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.129±0.025</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD. vs control, <sup>a</sup>P<0.05; vs. group BSA, <sup>b</sup>P<0.05; vs. control, <sup>c</sup>P<0.01, vs. BSA, <sup>d</sup>P<0.01. BSA, bovine serum albumin; AGE, advanced glycation end-product; SD, standard deviation; OD, optical density; Aβ, β-amyloid.

Changes in RAGE mRNA expression in cells intervened by α-LA (0.1 g/l). The cells in the AGE-BSA (8 µg/ml) group were chosen. In the α-LA (0.1 g/l) intervention group, there were no evident changes in APP mRNA as compared with the control group (Table IX and Fig. 3).
NIU et al.: α-LIPOIC ACID GREATLY ALLEVIATES THE TOXIC EFFECT OF AGEs

was no evidence of any changes in APP mRNA expression as compared with the control group (Table X and Fig. 4).

Discussion

Recent studies showed that AGEs play a very important role in the pathogenic process of neurodegenerative diseases, and such studies mainly focused on the direct toxic effect of AGEs on neurons. Sasaki et al found that there were many AGEs deposited in SP, NFTS, microglial cells and astrocytes in the brain tissue of AD patients (18). It was also indicated that AGE precursors such as methylglyoxal, glyoxal and HNE displayed cytotoxicity independently of Aβ, and further promoted Aβ aggregation (16). Takeuchi et al employed chicken egg albumin and BSA-AGEs to treat SH-SY5Y cells, primary astrocytes and N9 cells, which led to cell death (17).

The neurotoxic mechanism of AGEs remains unclear. It was reported that AGEs could indirectly modify Aβ in SP and tau protein in NFTS, and that the protein modified by AGEs were cytotoxic, a property that increased with the extent of modification (19). However, the inflammatory response generated by glycosylated Aβ simulating the microglial cell was much more severe than that of non-glycosylated Aβ (20). In vitro, Aβ formed a core with AGEs, which provoked additional aggregation by soluble Aβ. The unmodified Aβ as signal combined with the receptor for AGEs induced gene expression and aggravated the glycosylated process. The modified tau protein could cause abnormal phosphorylation and cross-linking and significantly decreased

Figure 2. Expression of RAGE mRNA in SH-SY5Y cells treated by different concentrations of BSA and AGE-BSA. BSA, bovine serum albumin; AGE, advanced glycation end-product; RAGE, receptor for AGE.

Figure 3. Levels of APP mRNA expression in SH-SY5Y cells that were intervened by LA before treatment with 80 µg/ml AGE-BSA. BSA, bovine serum albumin; AGE, advanced glycation end-product; APP, amyloid precursor protein; LA, lipoic acid.

Figure 4. Levels of RAGE mRNA expression in SH-SY5Y cells that were intervened by LA before treatment with 80 µg/ml AGE-BSA. BSA, bovine serum albumin; AGE, advanced glycation end-product; RAGE, receptor for AGE; LA, lipoic acid.
manner, suggesting increased formation of Aβ is involved in
tive stress. Misonou
which resulted in the generation of many cytokines and oxida
and further activated intracellular signal transduction pathway,
ons the surface of neurogliocytes. It was combined with AGEs
Maczurek
mRNA expression. It was also reported that the toxic effect of
increased formation of Aβ when introduced into the neuron.
MAP-tau would result in increased APP expression and
commonly seen in AD. It was reported that AGE-modified
microtubule assembly, further promoting pathologic changes
commonly seen in AD. It was reported that AGE-modified
MAP-tau would result in increased APP expression and
increased formation of Aβ when introduced into the neuron. However, in our study, it was demonstrated that AGE-BSA also
directly improved Aβ formation in a concentration-dependent
manner, suggesting increased formation of Aβ is involved in
the cytotoxicity of AGES. Moreover, it was also revealed that
there was no clear alteration of APP mRNA expression in
SH-SY5Y cells at 48 h after being treated by different protein
concentrations (20, 40 and 80 μg/ml) in the RT-PCR test. It was
speculated that the increased formation of Aβ was not caused
by an increase in APP expression as a result of increased APP
mRNA expression. It was also reported that the toxic effect of
AGES were predominantly mediated by oxidative stress (21).
Mazurek et al (22) found that there was AGE-specific receptor
on the surface of neurogliocytes. It was combined with AGES
and further activated intracellular signal transduction pathway,
which resulted in the generation of many cytokines and oxida-
tive stress. Misonou et al (23) used H2O2 to study the effect of
oxidative stress on SH-SY5Y cells, and found that it decreased
COOH-terminal fragments in the β-secretase pathway. Cho et al (24) proved that the RAGE signaling pathway
could enhance BACEI expression by activating NFαB both
in vitro and in vivo, further causing increases in Aβ formation.
Furthermore, AGE-RAGE could play a part by inducing CTGF
expression, and not necessarily by oxidative stress (25,26).
In addition, we also confirmed that AGE-BSA induced
increases in RAGE mRNA expression in SH-SY5Y cells.

### Table IX. Levels of APP mRNA expression in SH-SY5Y cells
that were intervened by LA before treatment with 80 μg/ml
AGE-BSA.

<table>
<thead>
<tr>
<th>Groups (g/l)</th>
<th>APP mRNA/actin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 μg/ml AGE-BSA</td>
<td>0.278±0.030</td>
</tr>
<tr>
<td>0.1 g/l α-LA</td>
<td>0.281±0.027</td>
</tr>
<tr>
<td>Control</td>
<td>0.284±0.033</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD. vs. control, *P>0.05. BSA, bovine serum albumin; AGE, advanced glycation end-product; SD, standard deviation; APP, amyloid precursor protein; LA, lipoic acid.

### Table X. Levels of RAGE mRNA expression in SH-SY5Y cells
intervened by LA before treatment with 80 μg/ml AGE-BSA
(mean ± SD).

<table>
<thead>
<tr>
<th>Groups (g/l)</th>
<th>RAGE mRNA/actin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 μg/ml AGE-BSA</td>
<td>0.591±0.040</td>
</tr>
<tr>
<td>0.1 g/l α-LA</td>
<td>0.543±0.030</td>
</tr>
<tr>
<td>Control</td>
<td>0.49±0.017</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD. vs. control, *P<0.05. BSA, bovine serum albumin; AGE, advanced glycation end-product; SD, standard deviation; LA, lipoic acid.

microtubule assembly, further promoting pathologic changes
commonly seen in AD. It was reported that AGE-modified
MAP-tau would result in increased APP expression and
increased formation of Aβ when introduced into the neuron. However, in our study, it was demonstrated that AGE-BSA also
directly improved Aβ formation in a concentration-dependent
manner, suggesting increased formation of Aβ is involved in
the cytotoxicity of AGES. Moreover, it was also revealed that
there was no clear alteration of APP mRNA expression in
SH-SY5Y cells at 48 h after being treated by different protein
concentrations (20, 40 and 80 μg/ml) in the RT-PCR test. It was
speculated that the increased formation of Aβ was not caused
by an increase in APP expression as a result of increased APP
mRNA expression. It was also reported that the toxic effect of
AGES were predominantly mediated by oxidative stress (21).
Mazurek et al (22) found that there was AGE-specific receptor
on the surface of neurogliocytes. It was combined with AGES
and further activated intracellular signal transduction pathway,
which resulted in the generation of many cytokines and oxida-
tive stress. Misonou et al (23) used H2O2 to study the effect of
oxidative stress on SH-SY5Y cells, and found that it decreased
COOH-terminal fragments in the β-secretase pathway. Cho et al (24) proved that the RAGE signaling pathway
could enhance BACEI expression by activating NFαB both
in vitro and in vivo, further causing increases in Aβ formation.
Furthermore, AGE-RAGE could play a part by inducing CTGF
expression, and not necessarily by oxidative stress (25,26).
In addition, we also confirmed that AGE-BSA induced
increases in RAGE mRNA expression in SH-SY5Y cells.

RAGE is the receptor of Aβ, and its expression would be
increased in the abundant Aβ environment (27). It was also
combined with Aβ at the nanomole level and amplified the
neuronal damage that was mediated by Aβ (28). In the in vitro
study, the combination of Aβ and RAGE could activate
NF-κB and simulate secretion of macrophage colony stimu-
lating factor (M-CSF) and IL-6, suggesting that Aβ-RAGE
played a very important role in the neuronal inflammatory
response (29). A prior study found that in a transgenic rat
model that expressed both neuronal RAGE and APP showed
evident cognitive disorder and decreased axonal conduction
ability as compared the rat with normal expression of neuronal
RAGE or APP in three to four month-old rats. At this time, the
rat showed no characteristic pathological changes consistent
with AD, indicating that the activation of RAGE by Aβ could
involve neuronal damage in the early stages of AD (6).

In our study, intracellular Aβ expression was evidently
increased after being treated by AGE-BSA, suggesting
increased formation of Aβ. RAGE mRNA expression was
increased, which was seen after Aβ combined with neurons
and RAGE expression being evident on the cytoplasmic
membranes of oligodendroglia cells. Then, NF-κB was initi-
at resulted in a persistent inflammatory response, neuronal
cytotoxicity and cell death (30). However, in the environment
with abundant Aβ, the expression was persistently increased,
which amplified the pathogenic effect that was induced by
Aβ (31). Zlokovic (32) found that in the neurons of APP
transgenic mice, the expression of RAGE accelerated the
decline in cognitive function and dysfunction of neurons
that was induced by Aβ. RAGE on the surface of encephalic
vascular endothelial cells could assist Aβ to pass through
the blood-brain barrier in the peripheral circulation (33),
upregulate CCR5 expression, and stimulate T cells that
entered the endocardium, causing a systemic inflammatory
response (34). Above all, as the ligand of RAGE, Aβ could
mutually interact, and thereby play a major role in cytoto-
icity of AGE-BSA.

Moreover, since RAGE is the specific receptor of
AGES, it can mediate toxic effect of AGES in many chronic
diabetic complications. It was found that the combination of
AGES-RAGE also activated the NF-κB signaling pathway
in microglial cells and astrocytes (20), which was one of the
promoters that regulated RAGE expression (35). The activa-
tion of site 1 and 2 upregulated RAGE expression, and further
promoted the combination of AGES and RAGE.

In addition, such combination also activated many
pathways such as that of protein kinase C signaling, protein
tyrosine kinases, and P2IRAS, causing a coordinated cellular
damage response (36). In short, AGES possess evident toxicity
against nerve cells, directly leading to increased formation of
Aβ and neurodegeneration. In the context of a toxic effect, the
Aβ-RAGE combination, AGES-RAGE combination, Aβ and
RAGE could all be involved. The increased formation of Aβ
would not be realized by increased APP expression caused by
increased APP mRNA expression, the detailed mechanism
requires further exploration.

The α-LA with terminal concentrations of 0.01, 0.1 and 1 g/l
was employed to pre-treat SH-SY5Y cells for 30 min to study
the influence of α-LA on the toxic effects of nerve cells
caused by AGES. The results show that α-LA at a dose of
NIU et al.: α-Lipoic Acid Greatly Alleviates the Toxic Effect of AGEs

0.1 g/l significantly resisted the toxic effect of AGEs-BSA to SH-SY5Y. Compared with the corresponding control group, the MTT metabolic rate was significantly increased, LDH release was significantly decreased and cell morphology was greatly improved. It suggested that α-LA had a protective effect for nerve cells.

In our study, we found that LA had a protective effect on nerve cells but not in a dose-dependent manner. Therefore, we deduced that LA entered cells by a certain carrier, and when the carrier was saturating, the effect would not be increased as the carrier supply. Moreover, increased LA concentration provoked altered osmotic pressure and pH, directly influenced the external environment of incubated cells, and further influenced the effect of LA.

We chose α-LA with an optimal concentration of 0.1 g/l to pre-treat cells in the AGE-BSA (80 µg/ml) group for 30 min and then tested the influence of α-LA on intracellular Aβ expression, APP mRNA and TAGE mRNA expression profiles. We found that as compared with the corresponding AGEs-BSA treatment group, the intracellular Aβ expression significantly decreased, which suggested that α-LA was partly related to a decreased expression in Aβ, at least in the protection of SH-SY5Y cells (Fig. 5).

There was no evident change in APP mRNA expression, which suggested that decreased Aβ was not realized by decreased APP expression that was caused by decreased APP mRNA expression. Furthermore, the expression of TAGE mRNA was significantly reduced. The results indicated that using an anti-RAGE antibody to prevent the combination of RAGE and ligand, significantly reduced the generation of inflammatory cytokines and nitric oxide by microglial cells (37).

The RAGE antagonist, sRAGE (soluble RAGE) was used to treat diabetic rats that lacked Apo-E. In this mode, treatment inhibited development of early angiosclerosis (38). High doses of sRAGE to mouse would isolate Aβ in the plasms, and would thus decrease intracranial Aβ levels (31). It was deduced that the weakened combination of Aβ-RAGE and AGEs-RAGE were also involved in the protective effect of LA for nerve cells. AGEs decreased the intake of glucose into SH-SY5Y cells, which was related to the inactivation of the glucose transporter, as previously reported by Keller et al (ref.?) However, after LA enters cells, pyruvate dehydrogenase would be activated, which would thus increase aerobic metabolism of glucose. Besides, it also made the glucose transporter (GLUT1, GLUT3, and GLUT4) translocate to the cytoplasmic membrane by activating phosphatidylinositol-3. Therefore, α-LA possibly enabled neuronal cells to resist damage that was caused by AGEs-BSA via improving glucose metabolism. The effect of decreased Aβ formation on improving glycometabolism still requires further exploration.

In conclusion, we found that Aβ is the key factor for AD pathological mechanisms, which is also an important target for AD treatment. In addition, α-LA could reduce Aβ generation and inhibit Aβ aggregation, and it also demonstrated utility in absorbing and passing through the blood-brain barrier, thus playing a very important role in the central nervous system degenerative diseases. Thus, we believe that Aβ should have wide developmental utility in the future.
Acknowledgements
Not applicable.

Funding
No funding was received.

Availability of data and material
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
GN and JG carried out the studies, participated in collecting data, and drafted the manuscript. YT and KZ performed the experiments and analyzed the data, and drafted the manuscript. QX helped to design the studies and modified the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 41: 2855‑2864, 2018
30. Onyango IG, Tuttle JB and Bennett JP Jr: Altered intracellular
signaling and reduced viability of Alzheimer's disease neuronal
cybrids is reproduced by beta-amyloid peptide acting through
receptor for advanced glycation end products (RAGE). Mol Cell
31. Deane R, Singh I, Sagare AP, Bell RD, Ross NT, LaRue B,
RAGE-specific inhibitor reduces amyloid β-mediated brain
32. Zlokovic BV: Neurovascular mechanisms of Alzheimer's neuro-
and Folin M: Involvement of the receptor for advanced glycation-end
products (RAGE) in β-amyloid-induced toxic effects in rat cere-
bromicrovascular endothelial cells cultured in vitro. Int J Mol
34. Li M, Shang DS, Zhao WD, Tian L, Li B, Fang WG, Zhu L,
Man SM and Chen YH: Amyloid beta interaction with receptor
for advanced glycation end products up-regulates brain endo-
thelial CCR5 expression and promotes T cells crossing the
35. Wong A, Lüth HJ, Deuther-Conrad W, Dukic-Stefanovic S,
Gasic-Milenkovic J, Arendt T and Münch G: Advanced glycation
endproducts co-localize with inducible nitric oxide synthase in
36. Boulanger E, Dequiedt P and Wautier JL: Advanced glycosylation
(In French).
and Münch G: Induction of novel cytokines and chemokines by
advanced glycation endproducts determined with a cytometric
38. Anzai Y, Hayashi M, Fueki N, Kurata K and Ohyama T: Protracted
juvenile neuronal ceroid lipofuscinosis - an autopsy report and