Metabolic Brain Disease (2019) 34:1761–1770
https://doi.org/10.1007/s11011-019-00482-5

ORIGINAL ARTICLE

N2L, a novel lipoic acid-niacin dimer protects HT22 cells against β-amyloid peptide-induced damage through attenuating apoptosis

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Received: 28 January 2019 / Accepted: 9 August 2019 / Published online: 2 September 2019

Abstract

β-amyloid protein (Aβ) is thought to be the primary cause of the pathogenesis of Alzheimer's disease (AD). Niacin has been reported to have beneficial effects on AD. Previously, we synthesized a novel compound lipoicacid–niacin dimer (N2L) and revealed that it had potent blood-lipid regulation and antioxidative properties without aflushing effect. Given that lipid metabolism is also associated with AD, the present study aimed to investigate the neuroprotective effects of N2L on $A\beta_{1-42}$-induced cytotoxicity in HT22 cells. We found that N2L significantly attenuated cell apoptosis, MDA level, ROS content, and the mitochondrial membrane potential corruption induced by $A\beta_{1-42}$ in HT22 cells. In addition, the activities of SOD, GSH-px and CAT that were decreased by $A\beta_{1-42}$ were also restored by N2L. Furthermore, N2L reduced proapoptotic signaling by increasing the expression of anti-apoptotic Bcl-2 and decreasing the protein expression of both pro-apoptotic Bax and cleaved Caspase-3. Together, these findings indicate that N2L holds great potential for neuroprotection against $A\beta_{1-42}$-induced cytotoxicity via inhibition of oxidative stress and cell apoptosis, suggesting that N2L may be a promising agent for AD therapy.

Keywords N2L · Aβ1–42 · HT22 cell · Neuroprotection · Oxidative stress

Introduction

Alzheimer's disease (AD), also known as senile dementia, leads to mortality in elderly people (Forman et al. 2004; Nordberg 2004; Zheng et al. 2002). AD has become one of the most devastating diseases in the world. It is characterized by pathological deposition of β-amyloid (Aβ) plaques, neurofibrillary tangles (NFTs) and loss of neurons (Selkoe 2002). Many studies have suggested that changes in $A\beta_{1-42}$ physicochemical properties and concentration potentially trigger its transition from physiological to pathological (Selkoe 2008). $A\beta_{1-42}$ has a capacity to cause a significant elevation in cytosolic reactive oxygen species and lipid peroxidation in primary cortical mouse neurons (Gunn et al. 2016). Moreover, intracerebroventricular (ICV) injection of $A\beta_{25-35}$ has been reported to change brain histology (Alkam et al. 2010; Meunier et al. 2006) and

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hippocampal function through inducing oxidative stress in animals (Trubetskaya et al. 2003). The abnormal deposition of Aβ in the brain plays an important role in the pathological mechanisms of AD, which include oxidative stress, mitochondrial diffusion, and excitotoxicity (Han et al. 2016). In addition, considerable evidence suggests that lipid peroxidation is involved in AD (Chang and Chang 2017; El Gaamouch et al. 2016; Liu and Zhang 2014). Antioxidant, antiapoptotic and hypolipidemic drugs may ameliorate AD (Frisardi et al. 2010; Schenk 2004; Thomas et al. 2001; Yamada and Nabeshima 2000). However, a single-targeted drug may not be sufficient to treat AD, a central nervous system (CNS) disorder involving multiple factors (Shen et al. 2015). Thus, a multifunctional drug with two or more targets may provide a better therapeutic effect against AD than a single-targeted one.

Metabolic disorder may increase the risk of AD. High-fat, high-glucose, and/or high-cholesterol diet may enhance the incidence of AD in later life due to the metabolic dysfunction that is often induced by such an unhealthy dietary habits (Zhu et al. 2018). Some studies have found that hyperlipidemia aggravates Aβ deposition and Tau phosphorylation by inducing oxidative stress (Rojo et al. 2017).

Alpha-lipoic acid (LA) is a "universal antioxidant" that has been used as a therapeutic agent for many chronic diseases, including diabetes mellitus and its associated peripheral neuropathy. LA has beneficial effects in the treatment of AD. It could easily penetrate the blood-brain barrier and moderate improve cognitive functions (Farr et al. 2003; Hager et al. 2001; Quinn et al. 2007; Zhang et al. 2018), and restore glucose metabolism and synaptic plasticity in a triple transgenic mouse model of AD (Sancheti et al. 2013; Sancheti et al. 2014).

Niacin (also named vitamin B3) and its related derivatives have shown neuroprotective effects in the previous studies (Lin et al. 2004; Rabie et al. 2010; Shehadah et al. 2010). AD can be caused by severe niacin insufficiency, and higher dietary intake of niacin or other B vitamins may protect against AD and age-related cognitive decline (Kim et al. 2014; Morris et al. 2004; Smith and Refsum 2016). However, niacin often provokes flushing reaction, which limits its usage (Kei and Elisaf 2012). Considering the biocompatibility and safety of physiological lipids composition, we combined LA and niacin into a novel compound lipoic acid-niacin dimer (N2L) which is a potent blood-lipid regulator without flushing effects (see Fig. 1a) (Pi et al. 2011). In our previous studies, N2L prevented L-glutamate-induced cytotoxicity and exerted protective effects against acrolein-induced human retinal pigment epithelium-19(ARPE-19) cell damage (Wang et al. 2013). Therefore, we hypothesized that N2L may be beneficial in the treatment of AD. The effects of N2L on neurotoxicity of HT22 cells induced by Aβ and its mechanisms was investigated.

Materials and methods

Materials

N2L (purity >95%) was synthesized as described previously (Chen et al. 2014), and N2L stock solution (100 mM) was dissolved in dimethyl sulfoxide (DMSO) and stored at −20 °C. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco-BRL (NY, USA). Poly-d-lysine, $A\beta_{1-42}$, 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA), and DMSO were obtained from Sigma-Aldrich Inc. (St Louis, MO, USA). The kits for malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and lactate dehydrogenase (LDH) were provided by the Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). A BCA protein assay kit, Hochest33258 and a mitochondrial membrane potential assay kit with JC-1 were purchased from the Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). Polyclonal antibodies against β-actin, cleaved caspase-3, Bax and Bcl-2 were obtained from Cell Signaling Technology (Danvers, MA, USA).

Cell culture

HT22 mouse hippocampal neuronal cells were maintained in DMEM supplemented with 10% (*v*/v) FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin and incubated at 37 °C under 5% CO2. HT22 cells were seeded on polyD-lysine-coated plates, and the medium was changed every 3 days.

MTT assay and LDH release assay

Cell viability was determined by MTT assay and lactate dihydrogenase (LDH) assay, as described in our previous paper (Luo et al. 2017). After treatment, 20 μL of supernatant per well were transferred into a 96-well microplate to determine LDH levels according to the manufacturer's instructions before adding MTT. Optical density was measured using a microplate reader (Bio-Tek, USA) at 405 nm. For the MTT assay, 10 μL of MTT (5 mg/mL) was then added to each well, and the mixture was incubated for 2 h at 37 °C. MTT reagent was then carefully replaced with DMSO (100 μL per well) to dissolve formazan crystals. After the mixture was shaken at room temperature for 10 min, absorbance was determined at 570 nm using a microplate reader (Bio-Tek, USA). Results were expressed as the percentage of the absorbance of control cells, which was set at 100%.

Morphological changes

HT22 cells grown on 48-well plates were treated with N2L and/or $A\beta_{1-42}$. Cells were then fixed with 4% paraformaldehyde and

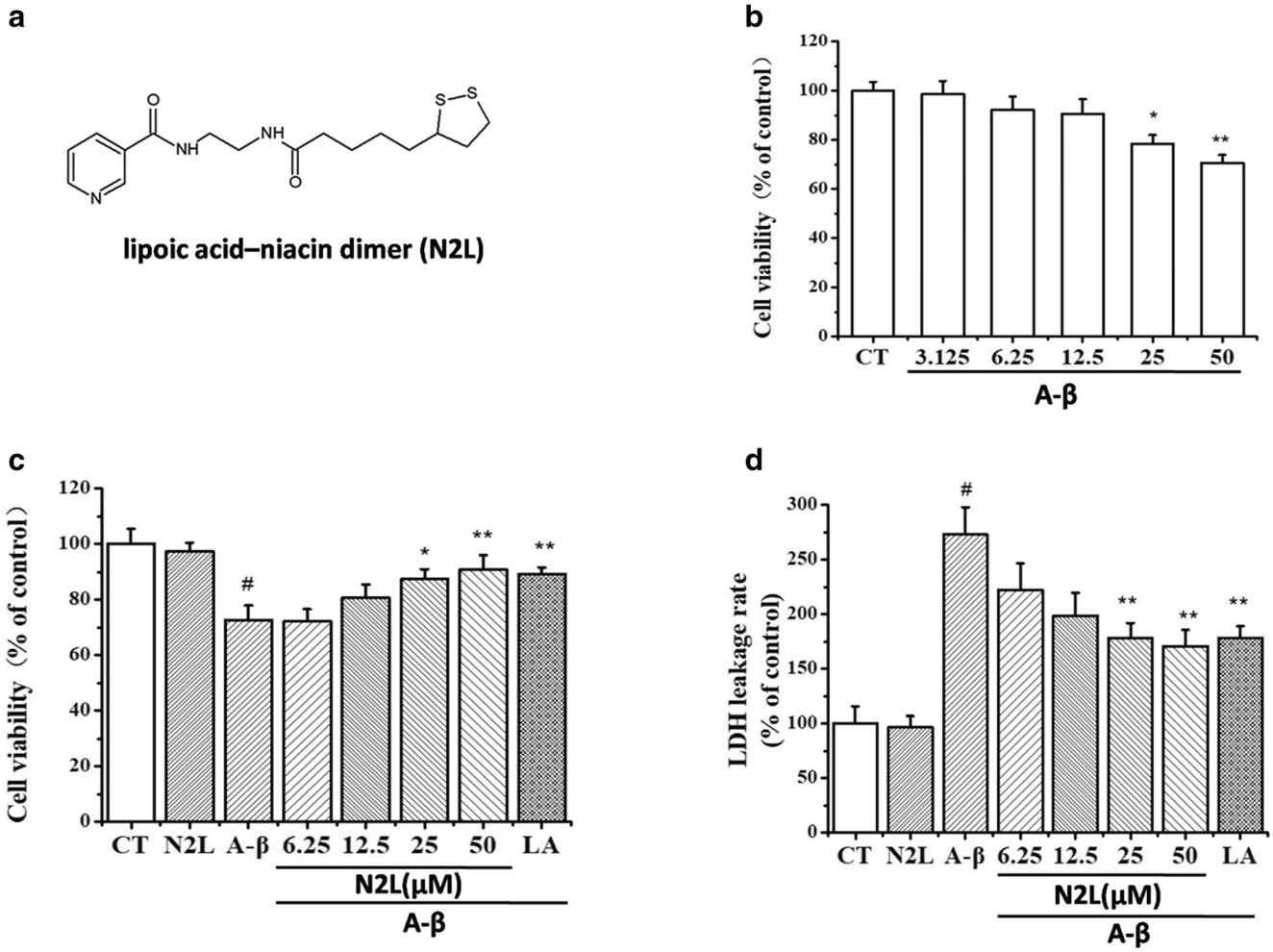

Fig. 1 Protective effect of N2L against $A\beta_{1–42}$-induced cytotoxicity in HT22 cells. (**a**) Chemical structure of N2L; (**b**) Cells were exposed to different concentrations of $A\beta_{1–42}$ (**c**)HT22 cells were treated with N2L (6.25–50 μM) for 2 h and then incubated with or without 50 μM $A\beta_{1–42}$ for a further 24 h. Cell viability was determined by MTT and LDH release (**d**) assays. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the control group (no $A\beta_{1–42}$); $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the $A\beta_{1–42}$-induced group

stained with Hoechst 33258 (5 μg/mL) for 10 min at 37 °C in the dark. Then Hoechst 33258 was removed by washing with PBS, morphological changes were observed via phase-contrast microscopy, and cell images were taken using a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

MMP determination

Mitochondrial membrane potential (MMP) was analyzed by using a fluorescent dye: JC-1. JC-1 penetrates cells and healthy mitochondria. At low membrane potentials (apoptotic cells), JC-1 exists as a monomer that emits green fluorescence. JC-1 aggregates and emits red fluorescence at higher membrane potentials (non-apoptotic cells). Assays were initiated by incubating HT22 cells with JC-1 (5 mg/L) for 20 min at 37 °C in the dark, and the fluorescence of separated cells was captured using inverted fluorescence microscopy (Olympus, Japan, at wavelengths of 490 nm excitation and 530 nm emission for green, and at 540 nm excitation and 590 nm emission for red). The ratios of red/green fluorescence were calculated.

Measurement of ROS

The intracellular ROS formation was measured by fluorescence using DCFH-DA. After treatment, cells were washed in serum-free medium at 37 °C in the dark and stained with 10 μM DCFH-DA for 30 min. The fluorescence from the DCF was analyzed using a fluorescence plate reader (Flex Station3, Molecular Devices, USA) at excitation and emission wavelengths of 488 and 525 nm, respectively, and images were taken using a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

Measurement of lipid peroxidation and antioxidant enzyme activities

The malondialdehyde (MDA, an index of lipid peroxidation) content and antioxidants [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px)] were tested using a commercial assay kit according to the manufacturer's instructions. HT22 cells were seeded in six-well plates at a density of 4×10^5 cells/well and incubated overnight. The cells were pre-incubated with N2L at different concentrations (12.5, 25, and 50 μM) for 2 h and then insulted with 50 μM $A\beta_{1–42}$ for 12 h. At the end of the treatment, the cultures were washed with ice-cold PBS and then pooled in 0.1 M PBS-0.05 mM EDTA-buffered solution and homogenized. The homogenate was centrifuged at 4000 g for 10 min at 4 °C, after which the protein concentration was determined by the BCA protein assay kit using bovine serum albumin (BSA) as a reference standard. The supernates were collected and stored at 80 °C until use.

Western blotting analysis

Western blotting analysis was performed as previously described (Wang et al. 2015). Cells from different experimental conditions or the hippocampus were lysed with ice-cold RIPA lysis buffer, and protein concentration was determined with a BCA protein assay kit according to the manufacturer's instructions. Equal amounts of lysate protein (20 μg/lane) were subjected to SDS-PAGE with 10% polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes. After transfer, the nitrocellulose blots were first blocked with 3% bovine serum albumin (BSA) in PBST buffer (PBS with 0.01% Tween 20, PH 7.4), and incubated overnight at 4 °C with primary antibodies in PBST containing 1% BSA. Immunoreactivity was measured by sequential incubation with horseradish peroxidase-conjugated secondary antibodies and detected by the enhanced chemiluminescence technique.

Statistical analysis

Data are expressed as the mean ± SEM for 3–5 independent experiments. The statistical significance of differences between the mean values for the treatment groups was analyzed with one-way or two-way analysis of Variance (ANOVA) followed by Dunnet *t*-tests using the software SPSS 13.0 (Chicago, USA). $P < 0.05$ was considered statistically significant.

Results

N2L prevents HT22 cells against $A\beta_{1–42}$ toxicity

To examine $A\beta_{1–42}$ -induced toxicity, HT22 cells were incubated with various concentrations of $A\beta_{1–42}$ for 24 h, as shown in Fig. 1b, treatment of $A\beta_{1–42}$ significantly decreased the cell viability of HT22 cells in a concentration-dependent manner, as compared to the control group, $A\beta_{1–42}$ at 50 μM caused about 30% decrease in cell viability, and therefore we used this concentration to HT22 cell in the following experiment. To evaluate the protective effects of N2L, HT22 cells were pretreated with PRI at indicated concentrations and then were incubated with or without 50 μM $A\beta_{1–42}$ for 24 h. MTT assay revealed that about 27.34% of cell viability was lost after exposure to $A\beta_{1–42}$(50 μM) for 24 h (Fig. 1b). N2L didn't induce cells death at 50 μM. Pretreatment of cells with different concentrations of N2L (6.25–50 μM) for 24 h, the cell viability was significantly increased in a concentration-dependent manner.

The protective activity of N2L was also confirmed by the LDH assay, as shown in Fig. 1c, $A\beta_{1–42}$ induced a significantly increase of the LDH release rate, which was increased to 238.66% of the control value. Pretreatment with N2L for 12 h markedly reduced $A\beta_{1–42}$-induced LDH release rate in a dose-dependent manner. The maximal effect appeared at 50 μM, so 50 μM was chosen for the subsequent experiments. To determine whether N2L blocked apoptosis induced by $A\beta_{1–42}$, the nuclear condensation was evaluated by DNA staining with Hoechst 33258. Nuclear condensation was induced by $A\beta_{1–42}$, which is a typical feature of HT22 cell apoptosis. The number of apoptotic bodies and the cells with nuclear condensation were decreased significantly by N2L (50 μM) (Fig. 2a, second row).

The reduction of MMP is a typical change induced by apoptosis (Ly et al. 2003). We investigated MMP by JC-1 assay (Fig. 2a, third row). The MMP assay was performed with HT22 cells incubated with JC-1 (5 mg/L) at 37 °C for 20 min. After HT22 cells were treated with 50 μM $A\beta_{1–42}$ for 24 h, the level of MMP decreased to 21.94% of its value in the absence of $A\beta_{1–42}$. N2L significantly increased MMP level (up to 39.34% of the control value).

Effect of N2L on oxidative stress and antioxidant enzyme activities in $A\beta_{1–42}$-treated HT22 cells

To investigate the effect of N2L on oxidative stress in $A\beta_{1–42}$-treated HT22 cells, we measured the level of intracellular ROS and MDA. As shown in Fig. 3a-c, after incubation with 50 μM $A\beta_{1–42}$ for 12 h, ROS production and MDA level increased to 174.97% and 161.94% of the control values, respectively. Moreover, pretreatment with N2L (50 μM) significantly reduced the intracellular ROS level (130.32% of the control value). N2L significantly decreased the MDA level in a dose-dependent manner (123.01% and 110.35% of the control value, respectively).

Oxidative stress is the product of out-of-balance between ROS and the antioxidant defense system. To determine whether the protective effect of N2L is associated with the activity of anti-oxidative enzymes, the levels of total SOD, CAT and

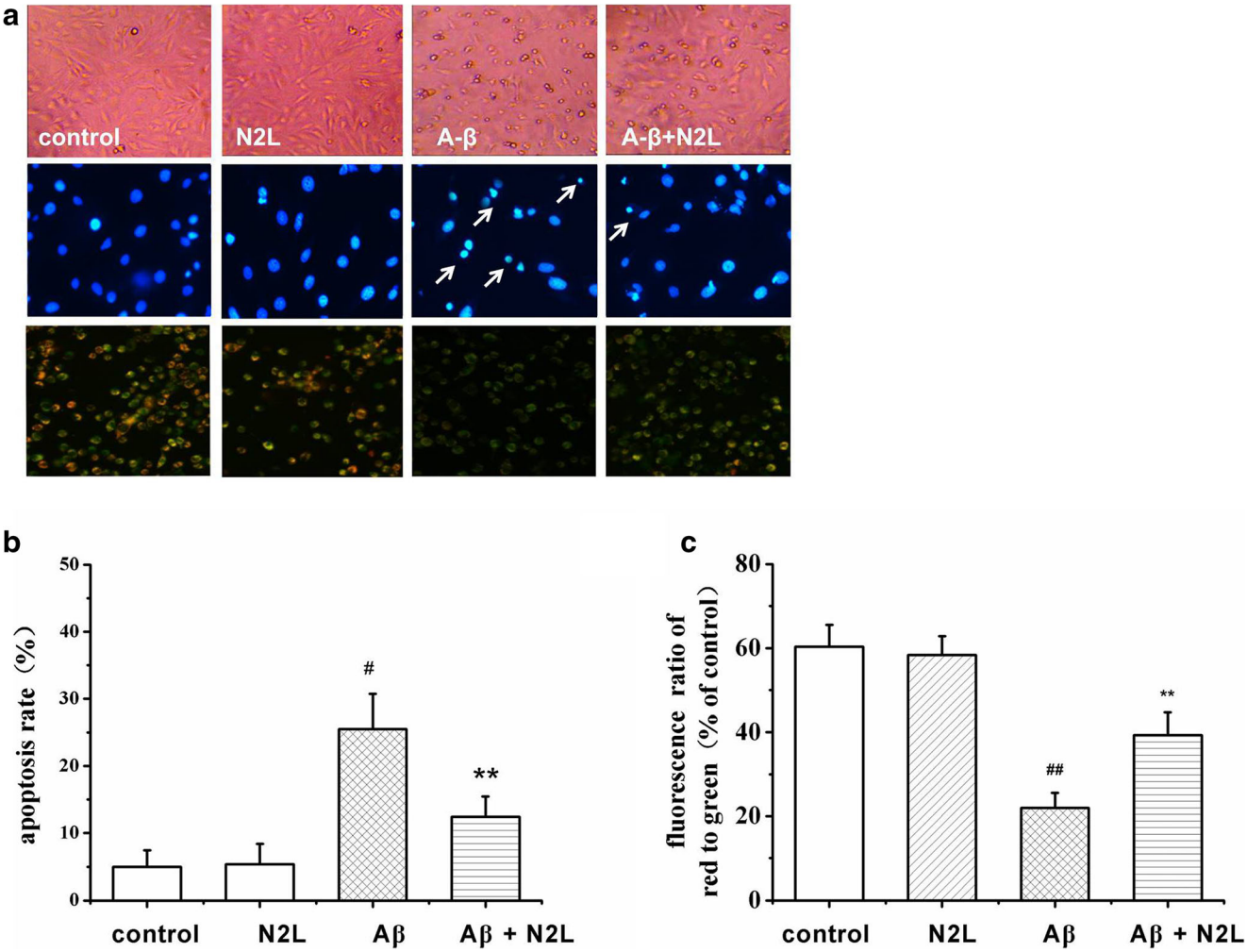

Fig. 2 $A\beta_{1–42}$ induced nuclear condensation, mitochondrial membrane morphological changes, and potential reduction, all activities that were inhibited by N2L. Pretreatment of HT22 cells with or without 50 μM of N2L for 2 h was followed by treatment with or without 50 μM $A\beta_{1–42}$ for 24 h. **a** The mitochondrial membrane potential decreased and nuclear agglutination and morphological changes induced by $A\beta_{1–42}$ were significantly reduced by N2L. The nuclear condensation and mitochondrial membrane potential were determined by Hochest33258 and JC-1 staining, respectively. Representative images were taken by a fluorescence microscope. The images shown are representative results of 3 independent experiments. The arrow indicates nuclear fragmentation or chromatin condensation. **b** A histogram showing the percentage of apoptosis in HT22 cells. **c** A histogram showing the fluorescence ratio of red to green. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the control group (no $A\beta_{1–42}$); $^{**}P < 0.01$ compared with the $A\beta_{1–42}$-induced group ($n = 6$)

GSH-Px were measured using a commercial assay kit. As shown in Fig. 3d, after incubation with $A\beta_{1–42}$(50 μM) for 12 h, the levels of SOD, CAT and GSH-Px were decreased to 41.94%, 71.93% and 51.55% of the control values, respectively. However, pretreatment with different concentrations of N2L increased the SOD level (70.07% to 83.35%), the CAT level (90.01% to 93.35%) and the GSH-Px level (73.11% to 80.44%) in comparison to cells treated with $A\beta_{1–42}$.

Effect of N2L on apoptosis-related protein changes in $A\beta_{1–42}$-treated HT22 cells

To further investigate whether apoptosis-related proteins were involved in the neuroprotective effect of N2L, Bcl-2, Bax and Caspase-3 levels were measured by western blot in HT22 cells. As we expected, $A\beta_{1–42}$ significantly increased the protein level of cleaved caspase-3 (147.39% of the control value), and N2L effectively blocked the activated Caspase-3 expression induced by $A\beta_{1–42}$ (Fig. 4a, b). Moreover, $A\beta_{1–42}$ increased the expression of Bax, whereas it decreased the expression of Bcl-2 (Fig. 4c); the Bcl-2/Bax ratio was decreased to 65.39% of that of the control group (Fig. 4d). On the other hand, N2L (50 μM) increased the Bcl-2/Bax ratio by 88.43% compared to that of the $A\beta_{1–42}$-treated group.

Discussion

AD is the most common form of dementia and the main cause of morbidity and mortality in the elderly. AD can be caused by

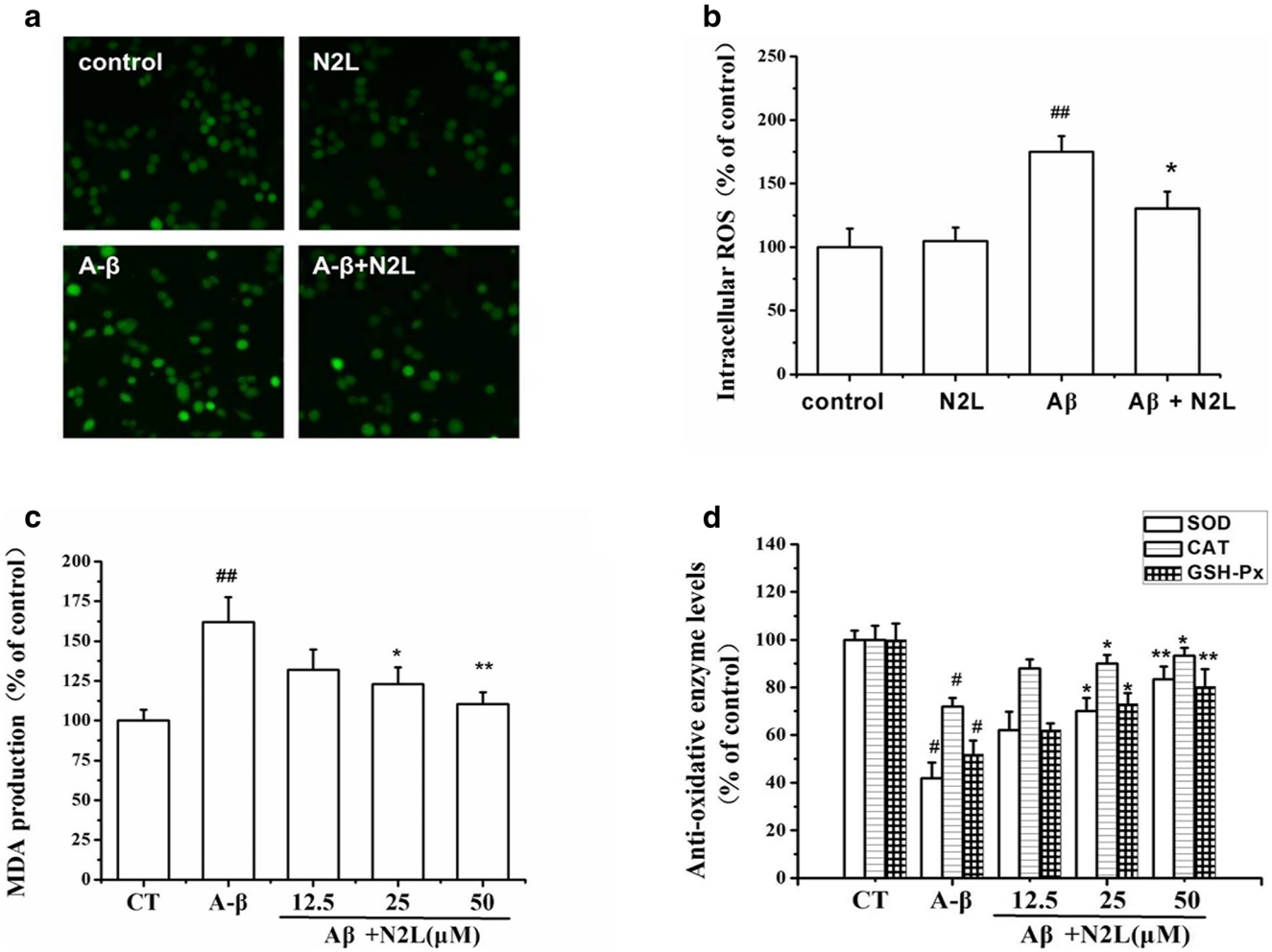

Fig. 3 Effect of N2L on $A\beta_{1–42}$-induced oxidative stress and anti-oxidative enzymes levels in HT22 cells. Cells were pretreated with different concentrations of N2L for 2 h, then cells were treated with or without 50 μM $A\beta_{1–42}$ for another 12 h. (**a**) ROS formation was measured with fluorescence microscope (200×) (insets): (**a**) control, (**b**) 50 μM N2L, (c) 50 μM $A\beta_{1–42}$ treated alone, (d) 50 μM $A\beta_{1–42}$ + 50 μM N2L. (**b**) ROS levels were measured with a fluorescence plate reader. The results are expressed as the percentage of values in the untreated control group (mean ± SD; $n = 3$). (**c**) MDA content was measured with an MDA assay. (D) SOD, CAT, and GSH-Px levels were measured using a standard assay. $^{\#}P < 0.05$ compared with the control group (no $A\beta_{1–42}$); $^{\#\#}P < 0.01$ compared with the control group (no $A\beta_{1–42}$); $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the $A\beta_{1–42}$-induced group

severe niacin insufficiency, it has been reported that niacin intake from foods was inversely associated with AD (Morris et al. 2004). On the contrary, a higher intake of niacin throughout young adulthood was associated with better cognitive function (Qin et al. 2017). It seems that niacin is a potential drug for AD. But niacin has a common adverse reaction—the flushing effect, which limits the long-term use of niacin in AD. Therefore, we synthesized a new compound N2L and found that it is an effective compound with antioxidant activity but no flushing effect. In this study, we have investigated the effects of N2L on HT22 cell toxicity induced by $A\beta_{1–42}$. Our results showed that N2L attenuated Aβ-induced oxidative stress, mitochondrial membrane depolarization, and apoptosis. The data of our experiments showed that N2L blocks apoptosis by decreasing the level of cleaved Caspase-3 and increasing the ratio of Bcl-2 to Bax. N2L might be benefit to the therapy of AD.

Aβ is considered to be the main cause of AD. Aβ damages mitochondria in neurons and plays an important role in the development of AD (Ashley et al. 2006; Tillement et al. 2011). Induction of ROS release by dysfunctional mitochondria is thought to be the cause of oxidative stress in AD (Kong et al. 2014; Lee et al. 2016). Hence, the neurotoxicity of Aβ is deeply related to oxidative stress, which is mediated by ROS (Canevari et al. 2004). The studies related to the mechanism of AD and LA have revealed that LA can inhibit the formation of Aβ fibrils (fAβ) and the stabilization of preformed fAβ, as well as protect cultured hippocampal neurons against neurotoxicity induced by Aβ and iron/hydrogen peroxide (Lovell et al. 2003; Ono et al. 2006). LA may reduce free radical damage and

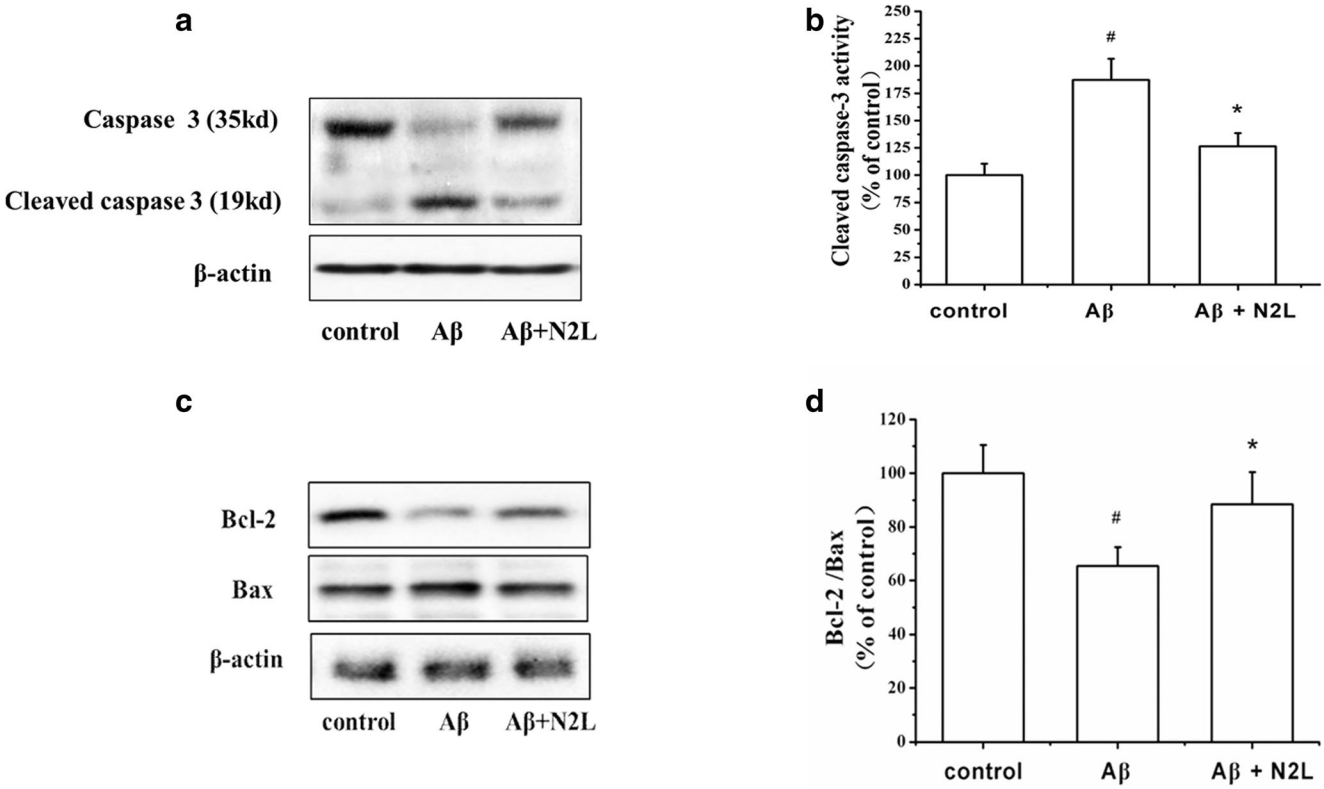

Fig. 4 N2L inhibited $A\beta_{1–42}$-induced mitochondrial apoptotic pathway in HT22 cells. Cells were pretreated with or without N2L (50 μM) for 2 h and then cultured in the presence or absence of 50 μM $A\beta_{1–42}$ for 12 h. Cell lysates were subjected to western blot analysis. (**a**)N2L inhibited $A\beta_{1–42}$-induced cleaved Caspase-3. (**b**) The amount of cleaved Caspase-3 was estimated by densitometric analysis of each protein band. (**c**)N2L prevented $A\beta_{1–42}$-induced changes in Bcl-2 family member expression. (**d**) The levels of Bax and Bcl-2 were quantified by densitometric analysis and the Bcl-2/Bax ratio was determined. Equal protein loading was confirmed by analysis of β-actin in the protein extracts. $^{\#}P < 0.05$ versus control group; $^{*}P < 0.05$ versus $A\beta_{1–42}$-treated group (n = 3)

attennuate inflammatory activities; thus, it may play a protective role in the neuronal ferroptosis, which is a recently discovered form of cell death dependent on iron and ROS (Xie et al. 2016; Zhang et al. 2018). Niacin inhibits vascular oxidative stress, redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells (Ganji et al. 2009); in addition, extended-release niacin therapy has been associated with decreased lipid oxidation of high-density lipoprotein in diabetic patients (Sorrentino et al. 2010). Niacin also improves mitochondrial metabolism and has been shown to reduce myocardial oxidative stress during kidney ischemia and reperfusion injury (Tai et al. 2015). The mechanism of niacin acting on AD is not clear at present. In the present study, we observed that $A\beta_{1–42}$ not only induced cell injury but also led to a high ROS level and a high level of mitochondrial dysfunction in HT22 cells. Furthermore, we found N2L attenuated cell apoptosis, accumulation of ROS and MDA level induced by $A\beta_{1–42}$, which indicated that the antioxidant effect of N2L might be helpful to reduce the apoptosis of neurons in AD.

It has been reported that ROS production is increased in AD patients. On the contrary, the levels of antioxidant enzymes (such as SOD, CAT, and GSH-Px) in AD patients were abnormally low. These low levels of antioxidant enzymes were also associated with the accelerated of neuronal death in AD patients induced by oxidative stress (Higgins et al. 2010; Padurariu et al. 2010). Consistent with the previous study, we also found that GSH-Px, CAT and SOD activity were significantly decreased by $A\beta_{1–42}$, and N2L significantly reversed the above process, suggesting that the neuroprotective mechanism of N2L depends at least in part on its interaction with the antioxidant system.

Mitochondrial dysfunction, including mitochondrial DNA damage and loss of protein in selective transport chain, has been found to play an important role in neuronal toxicity induced by Aβ. These results suggest that the dysfunction of mitochondria plays an important role in the pathogenesis and progression of AD, too (Abramov et al. 2004). In this study, we found that $A\beta_{1–42}$ can reduce MMP in HT22 cells, whereas N2L significantly inhibit the decrease of MMP induced by $A\beta_{1–42}$. Therefore, our findings provide further evidence that N2L exerted neuroprotective activity by preventing mitochondrial dysfunction.

Mitochondria deficiency triggers the mitochondrial apoptotic pathway, which plays a key role in the pathogenesis of AD (Onyango and Khan 2006). The proteins of Bcl-2 family and the ratio of the Bax/Bcl-2 play an important role in endogenous apoptotic pathway (Lee et al. 2012). Bax and Bcl-2 expression is inhibited by $A\beta_{1-42}$ toxicity, and this regulation is related with the increased activity of Caspase-3, which eventually serves as a key apoptotic effector of cell apoptosis (Yalcin et al. 2016). Niacin combined with selenium can alleviate the brain damage and improve neurological outcomes in rats with cardiac arrest; the effect was found to be due to up-regulation of DJ-1-Akt signaling and suppression of Caspase-3 cleavage (Kwon et al. 2018). In the present study, we found that $A\beta_{1-42}$ significantly decreased the ratio of Bcl-2 to Bax and increased the level of cleaved Caspase-3, both activities that were prevented by N2L, suggesting that the neuroprotective effect of N2L depend to some extent on its interaction with proteins of the Bcl-2 family. Poly (ADP-ribose)polymerase-1 (PARP-1) is the most active of the PARP enzymes, PARP-1 overactivation can cause neuroinflammation and cell death (Turunc Bayrakdar et al. 2014; Virag and Szabo 2002). Nicotinamide, an endogenous inhibitor of PARP-1, has been reported that it plays significant roles in cellular protection and in determining cellular fate in response to genotoxic DNA damage (Surjana et al. 2010). Recently, niacinamide as an amide of niacin therefore reduces the levels of oxidative stress, apoptosis, and PARP-1 activity in $A\beta_{1-42}$-induced rat model of Alzheimer's disease (Wohlrab and Kreft 2014). Hence, the relationship between PARP-1 inhibition and N2L related to cell apoptosis deserves to be investigated in future research.

It is well known that oxidative stress, inflammatory, and metabolic stress may precede proteinopathy in the precursor and early stages of sporadic AD (Galimberti and Scarpini 2011). Our experiments demonstrate that N2L, a novel lipoicacid-niacin dimer, has potent blood-lipid regulation and a significantly protective effect on $A\beta_{1-42}$- induced neuronal injury. It provides a new strategy to fight AD by protecting against proteotoxic, metabolic and oxidative stress. According to our results, the protective effect of N2L may be mediated through inhibiting oxidative stress and neuronal apoptosis. Therefore, the further studies are urgently needed to explore the effects of N2L against AD in vivo.

Acknowledgments This work was supported by the National Natural Science Foundation of China (No.81560662, No.81803536); Guangdong Provincial Key Laboratory of Construction Foundation (No.2017B030314030); Science and Technology Planning Project of Guangdong Province (No.2016A020226036, No 2016A020226011); Shenzhen Basic Research Projects (No.JCYJ20170818100811018) and Health and Family Planning Commission of Jiangxi Province (No.20185520).

Compliance with ethical standards

Disclosure statement There is no conflict of interests in this work.

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