Dendrobium alkaloids decrease the accumulation of Aβ through regulating α- and β-secretase of hippocampal neurons in SD rat

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Background Alzheimer's disease (AD) is the primary cause of dementia in the elderly. The imbalance between production and clearance of amyloid β peptides (Aβ) is a very early, often initiating factor in AD. *Dendrobium nobile* Lindl. Alkaloids (DNLA) extracted from a Chinese medicinal herb has been shown to have protective effect on neurons impairment.

Methods We exposed cultured hippocampus neurons to DNLA to investigate the effect on Aβ of DNLA *in vitro*. The assessment of cell viability was evaluated by MTT assay. Proteins were analyzed by Western blot.

Results As the result, cell viability of hippocampal neurons was not changed significantly after the treatment with DNLA. DNLA reduced the protein expression of amyloid precursor protein (APP), disintegrin and metalloprotease 10 (ADAM10), β-site APP cleaving enzyme 1 (BACE1) and $A\beta_{1\text{-}42}$ of hippocampal neurons in rat, increased the protein expression of ADAM17.

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Abstract

Background

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Keywords *Dendrobium nobile* Lindl. alkaloids; hippocampal neurons; α-secretase; β-secretase; γ-secretase

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease of the central nervous system that causes dementia in a large percentage of the aged population and for which there are only symptomatic treatments. Clinically, AD is typically characterized by progressive loss of memory, declining cognitive function, decreased physical function and ultimately death. The biology of AD is characterized by two major protein abnormalities in the brain of affected individuals: the extracellular accumulation of amyloid β (Aβ) plaques and intraneuronal deposits of neurofibrillary tangles (NFTs) (De-Paula et al. 2012). At present, the pathogenesis of AD has yet to be fully elucidated, and the main hypotheses proposed include deposition of Aβ protein, loss of choline neurons, abnormal activation of inflammatory reactions, disturbance of energy metabolism, genetic abnormalities and oxidative stress, etc. (Choudhry et al. 2012; Mouton-Liger et al. 2012; Riazantseva et al. 2012).In addition, environmental factors (Ashok et al. 2015; Banerjee et al. 2014; Cheng et al. 2015), diet (Choudhry et al. 2012)and diseases (Cansu et al. 2017; Faraco et al. 2016; Yousof Ali et al. 2015) also increase the risk of AD. Thereinto, it is widely accepted that the Aβ peptide cascade plays a critical role in the development of AD (Baranello et al. 2015; Kowalska 2004).

Aβ mainly consists of $A\beta_{1-40}$ and $A\beta_{1-42}$, and $A\beta_{1-42}$ is easier to polymerize in the brain for its stronger tendency of oligomerization. Thus, it has a strong neurotoxicity and hydrophobicity. According to the “amyloid cascade hypothesis” of AD, the imbalance between the production and clearance of Aβ is the basis of amyloid plaque formation that is the initial causes of AD (Baranello et al. 2015; Kowalska 2004; Selkoe & Hardy 2016). Under the normal physiological conditions, between the production and the clearance of Aβ and the value of $A\beta_{1-40}/A\beta_{1-42}$ maintains dynamic balance. Low concentration of Aβ monomers can enhance memory and keep synaptic plasticity. Thereby, Aβ plays a normal physiological function. Otherwise, in the pathological status, the mutation of presenilin and amyloid precursor protein (APP) gene in familial AD (FAD) affects the processing process of APP, causing excessive production of Aβ, especially $A\beta_{1-42}$. Once the dynamic balance is broken, $A\beta_{1-42}$ accumulates in large quantities. As a result, the accumulation of $A\beta_{1-42}$ forms "senile plaques" (SP), which has neurotoxic effects and damages neurons (Colombo et al. 2013; Kowalska 2004).

It is generally known that Aβ is generated by APP metabolism. APP in its mature form can be processed by at least two proteolytic pathways (Kowalska 2004), the so-called “non-amyloidogenic” and the “amyloidogenic” pathways. In the first pathway, α-secretases cleaves APP with the γ-secretases, thus impeding the formation of the toxic Aβ peptide. In the second one, Aβ, mainly to $A\beta_{1-40}$ and $A\beta_{1-42}$ amino acids long peptides, is produced in a two-step proteolytic process initiated by β-secretase and followed by γ-secretase. In brief, α-, β- and γ-secretase play key roles in the processing of APP. Being able to reduce the formation of the toxic Aβ is obviously an immediate approach in the trial to prevent AD. And it has been reported that the activation of α-secretase promotes the cleavage of APP, which can reduce the production of Aβ (Lichtenthaler 2012). And the inhibition of the activity of β - (Hilpert et al. 2013)and γ-secretase (Wolfe 2012) can also reduce the production of Aβ. So, targeting α-, β- and γ-secretase are the

promising focus of AD research.

The α-secretases (Lichtenthaler 2011) is comprised of some kinds of disintegrin and metalloprotease (ADAM), including ADAM9, ADAM10, ADAM17 and so on. These molecules work together to ensure its function as α-secretases. It is noteworthy that ADAM10 and ADAM17 are considered to be the most important α-secretase involved in the physiological processing of APP in the brain. β-secretase β-site APP cleaving enzyme (BACE) is a novel 501 amino acid type 1 transmembrane aspartic acid protease related to the pepsin and retroviral aspartic protease family, including N-terminal signal peptide and pro-peptide region, as well as the catalytic region of mature protein in the middle (46-460), transmembrane region and C-terminal tail (478-501). And BACE1 is a new subclass of I radix asparagi ammonia acyl proteinase, which is the most important β-secretase (Ebina et al. 2009). γ-secretase is an aspartyl intramembranous protease composed of presenilin (PSEN/PS), nicastrin (NCT), either anterior pharynx 1 (Aph1) and PSEN enhancer 2 (Pen2) with 19 transmembrane domains, and in a ratio of 1:1:1:1 (Cacquevel et al. 2012; Crump et al. 2013). PS1 as the catalytic core of γ- secretase, it can exert independent γ-secretase function (Woodruff et al. 2013). Therefore, it is significant to explore ADAM10, ADAM17, BACE1 and PS1 for the prevention and treatment of AD.

Dendrobium nobile Lindl. alkaloids (DNLA) was originally extracted from the traditional Chinese herbal medicine *Dendrobium nobile*. The chemical structures of these ingredients of DNLA were shown in the Figure. 1, and the chromatograms of the sample solutions were shown in the Fig. 1B (Nie et al. 2016). The previous studies indicated that DNLA can improve the neuronal disruption caused by lipopolysaccharide (Y. Li et al. 2011), and oxygen-glucose deprivation (Wang et al. 2010) and reperfusion, and decrease neuronal apoptosis, hyperphosphorylation of tau protein, and Aβ deposition in the rat brain (Nie et al. 2016). Furthermore, *in vitro* experiments, we found that DNLA could alleviate Aβ*25-35* induced axonal injury by improving autophagic flux in neurons (Li et al. 2017; Zhang et al. 2017). This study aims to investigated the effect of DNLA on Aβ and relative secretases of hippocampal neurons in rat, and further explore the mechanism underlying the regulation of the APP metabolism pathway.

Materials & Methods

Materials

Dendrobium was purchased from Xintian Traditional Chinese Medicine Industry Development Co., LTD., of Guizhou Province. DNLA was isolated from the extracts, and analyzed by LC MS/MS. Alkaloids accounted for 79.8% of the DNLA, and mainly contained 92.6% dendrobine ($C_{16}H_{25}O_2N$), 3.3% dendrobine-N-oxide ($C_{16}H_{25}O_3N$), 2.0% nobilonine ($C_{17}H_{27}O_3N$), 0.9% dendroxine ($C_{17}H_{25}O_3N$), 0.32% 6-hydroxy-nobilonine ($C_{17}H_{27}O_4N$), and 0.07% 13-hydroxy-14-oxodendrobine ($C_{16}H_{23}O_4N$) (Nie et al. 2016). The trypsin (SH30042.01), DMEM/F12 medium (SH3002301B) were purchased from

HyClone (America). FBS (S9030) was purchased from Solarbio (China). DES, Neurobasal A(A2477501), B27(17504044) were purchased from Gibco (America). The goat anti-mouse IgG H&L (Alexa Fluor® 488) was purchased from Life Technologies (America). Anti-beta III tubulin antibody(ab14545), goat anti-rabbit IgG H&L (ab150113), anti-ADAM10 (ab1997), anti-BACE1 (ab2007), anti- Presenilin 1/PS-1 (ab76083), anti-$A\beta_{1\text{-}42}$ (ab201060) were purchased from Abcam (America). Anti-APP (D260097) and anti-ADAM17 (D151531) were purchased from Sangon Biotech (China).

Animals

Sprague-Dawley (SD) rats (about 200-250 g) were purchased from the Laboratory Animal Center, Chongqing, China [Grade: specific pathogen-free (SPF), Certificate NO. SCXK 2012-0005] with 22–23°C, and a 12-hour light/dark cycle. 5 SD rats (male: female = 1:4) were housed in each cage to propagate the newborn SD rats and given food and water freely. All animal procedures were approved by the animal experimental ethical committee of Zunyi Medical University.

Pretreatment of DNLA

DNLA was soluble in dimethyl sulfoxide (DMSO) and was stored at −20°C. When using, it was diluted to different concentrations with Neurobasal A supplemented with 2% B27. The final concentration of DMSO was 0.01% (v / v) in our experimental system.

Culture of hippocampus primary neurons and identification

Hippocampus tissues were separated on ice from newborn SD rats born within 24 ~ 48 hours and then incubated in 0.125% of trypsin at 37°C with 5% CO_2 for 15 minutes. Hippocampus tissues were triturated by passing repeatedly through 1-mL pipette tip and filtered through sieve with 200, 300, 400 mesh, then cells were collected by centrifugation at 179 g for 8 minutes. Cells were seeded on poly-L-lysine-coated (4mg / mL) 6-well or 24-well plates or 96-well and cultured in DMEM / F12 medium supplemented with 10% FBS, 10% DES,100 U/mL penicillin and streptomycin at 37°C with 5% CO_2. After 4 hours, DMEM / F12 medium were replaced with Neurobasal A supplemented with 2% B27 for the duration of experiments. Changed the half-amount liquid every two or three days. On the 3th day, neurons were treated with cytarabine. On the 8th day, neurons in good condition were used for following experiments. The animal procedures were approved by the Animal Experimentation Ethics Committee of Zunyi Medical University. The profile of neurons was visualized by immunofluorescence staining using mouse monoclonal anti-beta III tubulin antibody (1:1000) and goat anti-mouse IgG H&L (1:1000). DAPI (1:20) was used to mark cell nucleus.

Assessment of cell viability by MTT assay

Neurons were seeded into 96-well plates and treated with DNLA for a desired time period at the indicated

concentrations. Five replicates were made for each treatment. After treatment, cell viability was evaluated by the MTT assay as previously described. The cell viability was expressed as a percentage of OD in cells with indicated treatments to that in cells with DMSO control treatment.

Western blot assay

Total protein was extracted from cultured neurons using a total protein extraction kit and quantified by BCA protein assay kit. Equal amounts of protein (20 μg) per lane were separated by SDS-PAGE gels and then transferred to a PVDF (0.45 μm) membrane. The membranes were incubated with the following primary antibodies: anti-APP (1:500), anti-ADAM10 (1:1000), anti-ADAM17 (1:1000), anti-BACE1 (1:1000), anti-PS1 (1:1000), anti-$A\beta_{1-42}$ (1:1000), β-actin (1:2000), and GAPDH (1:2000) at 4°C overnight, followed by incubation with secondary antibody at 4°C for 1 hour. The membranes were visualized using chemiluminescence reagent ECL Plus (E003-100). The image was scanned, and band densities were quantified using Quantity One 1D analysis software v4.52 (BioRad). GAPDH or β-actin was used to normalize protein loading.

Tatistical analysis

All data were expressed as mean ± SD and analyzed statistically by the SPSS 17.0 software. The normal distributed data firstly were analyzed statistically via one-way analysis of variance (ANOVA). $P < 0.05$ was considered to be statistically significant.

Results

The purity of neurons of hippocampal neurons in SD rat

First, we examined the purity of neurons by immunofluorescence staining, the result showed the neurons purity was, at least, 92.5% (Figure 2).

Effects of DNLA on the cell viability of hippocampal neurons in SD rat

The primary hippocampus neurons were treated with DNLA for 48 hours, we detected the viability of neurons in each group by MTT assay. The result showed that DNLA did not change significantly the cell vitality of hippocampal neurons (Figure 3).

DNLA decreased the accumulation of Aβ through decreasing APP

As mentioned above, it has been indicated that dementia is attributed to synaptic dysfunction and neuronal loss in the hippocampus and its associated cortex, which are caused by the accumulation of Aβ oligomers. We detect the protein expression of $A\beta_{1-42}$ and the result showed DNLA decreased the protein expression of $A\beta_{1-42}$ (Figure 4 B). In order to find out whether the effects of DNLA on the protein

expression of $A\beta_{1-42}$ in hippocampal neurons in SD rat was relative to APP, which being cleaved to $A\beta_{1-42}$ and the other Aβ fragment, we examined the protein expression of APP in hippocampus primary neurons. Furthermore, PS1 (γ- secretase) participates in both the "non-amyloidogenic" and the "amyloidogenic" pathways. Therefore, we also examined the protein expression of APP. As the Figure 4 shows, DNLA decrease the protein expression of APP in hippocampus primary neurons of rats, but did not change the protein expression of PS1.

DNLA decreased the accumulation of Aβ through regulating α-secretase

As a protective factor for the progress of AD, α-secretase plays a significant role in the "non-amyloidogenic" pathway. What's more, ADAM10 and ADAM17 are considered to be the most important α-secretase involved in the physiological processing of APP in the brain. We further probed the effect of DNLA on α-secretase (ADAM10 and ADAM17). Our results indicate that DNLA increased the protein expression of ADAM17, however, decreasing the protein expression of ADAM10 (Figure 5).

DNLA decreased accumulation of Aβ through inducing BACE1 (β-secretase)

Additionally, besides α-secretase, β-secretase, being the crucial factor in the "non-amyloidogenic" pathways, is relative to APP metabolism, which is the source of Aβ. BACE1 has been found to have important physiological roles in the progress of producing Aβ. Inhibiting the activity of BACE1 can reduce the accumulation of Aβ, which trigger the exacerbation of AD. We have confirmed DNLA could decrease the production of Aβ. Moreover, the reduction Aβ involved APP and α-secretase. We need to further confirm whether this is related to BACE1. As indicated in Figure 6. These findings indicated that DNLA could decrease the protein expression of BACE1 in hippocampal neurons in SD Rat.

Discussion

Learning and memory is the advanced functions of the nervous system and is the integration of nervous system functions. The hippocampus is closely related to learning and memory. The hippocampus, especially the entorhinal cortex of the hippocampus, is the place where AD first produces lesions. In this experiment, primary cultured hippocampal neurons of SD rats were used as experimental objects. Primary cultured neurons have not changed greatly due to their short body detachment, and to a certain extent, they reflect their true status in body. Previous studies suggested that primary hippocampal neurons cultured for 6 days have matured fully (Zhang et al. 2017). Our neurons, culturing for 8 days, for the research, had been mature and degenerate. During the period of 8 ~ 10 days, hippocampal neurons degenerate to more degenerate, as the human developing the old into older. With the treating of DNLA, our original intention is to hope that the process of fading will be delayed.

DNLA was exacted from *Dendrobium nobile* Lindl, which is a traditional Chinese medicinal

material and be produced in Chishui, Guizhou. As a pharmacological active ingredient of *Dendrobium nobile*, DNLA has protective effect on nervous system (Yu-peng 2017). Previous studies in the research group showed that APP, β-secretase and Aβ protein in hippocampus of transgenic mice Tg2576 were reduced (YIN 2013). Additionally, DNLA treatment can obviously improve the learning and memory of middle-aged APP/PS1 transgenic mice, and improve the learning and memory of wild type mice, suggesting that DNLA has potential health care value (Jingshan 2016; Nie et al. 2018). In this experiment, the neurons in normal growth state were treated with different concentrations of DNLA, and the effects of DNLA on the cell viability, APP, Aβ and its related secretase (α-, β- and γ- secretase) in normal state were preliminarily explored.

The senile plaque formed by extracellular deposition of Aβ is one of the main pathological symbol of AD. Moreover, with the increase of age, Aβ deposition increasing (Niedowicz et al. 2014). Aβ mainly includes $A\beta_{1-40}$ and $A\beta_{1-42}$. Among them, $A\beta_{1-42}$ has stronger neurotoxicity and hydrophobicity, and it has a strong tendency to oligomerize, so it is more easily polymerized in the brain. $A\beta_{1-42}$ accumulates in a large amount, forming “senile plaques”, which produces neurotoxic effects, which damage the neurons and cause damage. Thus, inhibiting the production of $A\beta_{1-42}$ can delay the pathogenesis of AD (Zhu et al. 2011). In this study, the protein expression of $A\beta_{1-42}$ in hippocampal neurons of SD rats was detected. The results showed that DNLA can reduce the protein expression of $A\beta_{1-42}$ in hippocampal neurons.

Tracing back to the original, APP is the precursor protein of Aβ and is a member of a family of related proteins, including amyloid precursor-like proteins (APLP1 and APLP2) in mammals and amyloid precursor protein-like (APPL) in drosophila, all with large extracellular structures. One-way transmembrane protein of the domain, but only APP produces amyloidosis fragments (O'Brien & Wong 2011). In theory, as the precursor protein of Aβ, if the APP protein could be reduced, the production of Aβ can be reduced from the source. In the present study, DNLA lower the protein expression of APP. Anyway, γ-secretase participants in the “non-amyloidogenic” and the “amyloidogenic” pathways, playing indispensable role. Some studies have suggested that the inhibition of the activity of γ-secretase could reduce the production of Aβ. γ-secretase contains PS (including PS1 and PS2), NCT, Pen-2 and Aph-1 and is also the rate-limiting enzyme of APP to produce Aβ (T. Li et al. 2011). A number of studies have shown that inhibition of γ-secretase activity is beneficial to reduce Aβ production (Wolfe 2012). As the core catalytic subunit of γ-secretase, PS1 is its main active component (Garcia-Ayllon et al. 2013) and has independent γ-secretase function (Woodruff et al. 2013). PS1 overexpression may be a risk factor for late-onset SAD (T. Li et al. 2011). Inhibition of PS1 expression can reduce Aβ production (Futai et al. 2016). In our study, the protein expression of PS1 have no changes significantly. The results show that DNLA did not transform PS1. $A\beta_{1-42}$ produced by the γ-secretase did not increase, but cut back. Therefore, we have logical reasons to believe that DNLA affect the process of APP metabolism to Aβ through other means. We had to explore it further.

Afterwards, to explore the effect of DNLA on the non-amyloidogenic pathway, we detect the α-

secretases, including the protein of ADAM10 and ADAM17. ADAM17 is an important α-secretase involved in the non-amyloidogenic pathway of APP. Enhancing the activity of ADAM17 can induce the increase in the secretion of a soluble sAPPα fragment with neuroprotection and a decrease in Aβ production. Therefore, ADAM17 is considered to be a potential therapeutic target for AD (Qian et al. 2016). The results of this experiment show that DNLA can increase the protein expression of ADAM17 and reduce the production of Aβ to a certain extent. ADAM10 is also an alpha-secretase that treats APP by non-amyloidogenic pathway, which cleaves APP to produce sAPPα and avoids the production of Aβ. Studies have also found an age-dependent increase in ADAM10 levels (Schuck et al. 2016). While, experiments by B. Shackleton et al. (Shackleton et al. 2016) suggested that inhibition of ADAM10 promoted the clearance of $A\beta_{1-40}$ and $A\beta_{1-42}$ in the brain of mice with AD, thereby, reducing the level of Aβ. This may be due to the fact that others α-secretase processes the APP when ADAM10 is reduced or absent. Other studies have reported that other members of the α-secretase family, such as ADAM9 and ADAM17, can compensate for the reduction in ADAM10 activity (Asai et al. 2003; Hartmann et al. 2002). It has also been reported that when ADAM10 is absent or reduced, sAPPα production is reduced or Aβ is significantly increased due to the lack of compensation for α-secretase (Kuhn et al. 2010; Postina et al. 2004; Suh et al. 2013). In addition, inhibition of BACE1 increased ADAM10 cleavage of APP, but decreased ADAM10 activity but increased the risk of AD by increasing β-secretase cleavage of APP (Colombo et al. 2013). In this experiment, the expression of ADAM10 protein in hippocampal neurons treated with DNLA was decreased, the expression of ADAM17 protein was increased, and the expression level of Aβ protein was decreased. It is suggested that DNLA can reduce the expression of ADAM10 protein, promote the clearance of Aβ and reduce the Aβ level in hippocampal neurons of SD rats.

In the amyloidogenic pathway, APP finally produces Aβ by β- and γ-secretase cleavage. β-secretase, including BACE1, cathepsin S (Cat S) and cathepsin L (Cat L) (Schechter & Ziv 2011), cathepsin D (cathepsin D, Cat D) and cathepsin B (cathepsin B, Cat B) (Zhou et al. 2012), is the rate-limiting enzyme of APP to produce Aβ. It has been suggested that BACE2 also belongs to β-secretase, but it was later proved to be a newly discovered η-secretase (Willem et al. 2015). Although Cat S, Cat L and Cat B may be further developed as targets for the treatment of AD (Schechter & Ziv 2011), the current main target for β-secretase remains BACE1. Numerous studies have shown that inhibition of BACE1 can reduce the production of Aβ (Adwan et al. 2014; Yan & Vassar 2014; Yun et al. 2013; Zhu et al. 2012). The results of this experiment showed that the expression of BACE1 protein was decreased after treatment with DNLA, suggesting that DNLA can reduce the protein expression of BACE1 in neurons, which is a probably reason for reducing the protein expression of $A\beta_{1-42}$, which is crucial to the development of AD.

Under the experimental conditions, there are some drawback in our study. We need further provide more direct evidence to reduce the Aβ with DNLA through affecting α- and β-secretase. In order to further explore the effects of DNLA on α-, β- and γ-secretase, the products of APP, such as sAPPα, P3, sAPPβ and CTF-γ, which were lysed by corresponding secretases, should be further tested to some extent

to explain the effect of DNLA on related secretases and its functions. In addition, if the activity of α-, β-, γ-secretase can be directly detected, the experiment will be more meaningful and convincing. Moreover, the reduction of Aβ is to reduce its source and promote its clearance. In this experiment, the expression of $A\beta_{1-42}$ protein in hippocampal neurons of SD rats is reduced, which is the result of various effects. Therefore, the exact mechanism of $A\beta_{1-42}$ reduction needs further study.

Conclusions

The results show that DNLA can decrease the $A\beta_{1-42}$ of hippocampal neurons in the rat via through regulating α- and β-secretase.

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Figure 1

Fig. 1. Chemical structures of DNLA.

(A) Dendrobine, (B) Dendrobine-N-oxide, (C) Nobilonine, (D) Dendroxine, (E) 6-Hydroxy-nobilonine, and (F) 13-Hydroxy-14-oxodendrobine.

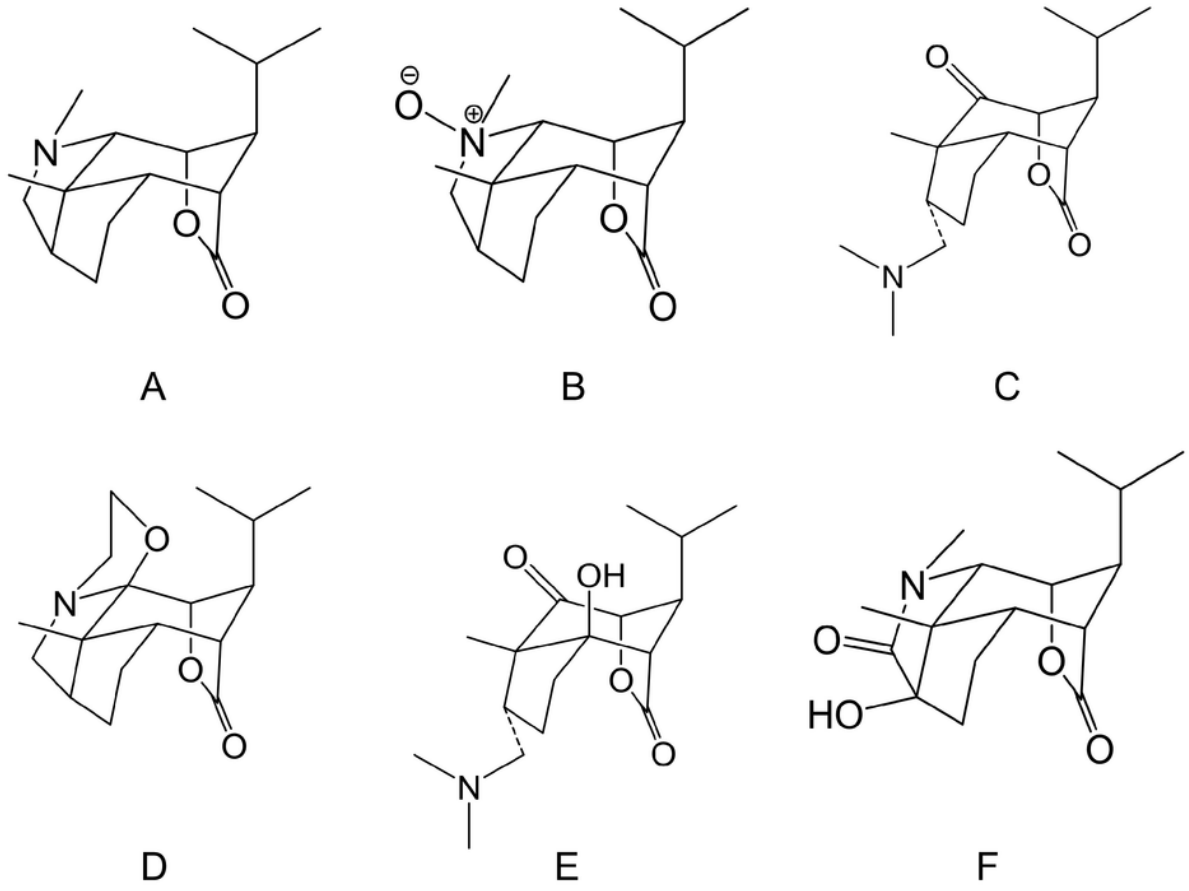

Figure 2

Fig. 2. The purity of neurons of hippocampal neurons in SD rat.

The primary hippocampus neurons have been cultured for 8days before treating with DNLA. (A) Neurons purity was detected by immunofluorescence technique. (B) Neurons purity account for more than 92.5% (n = 3).

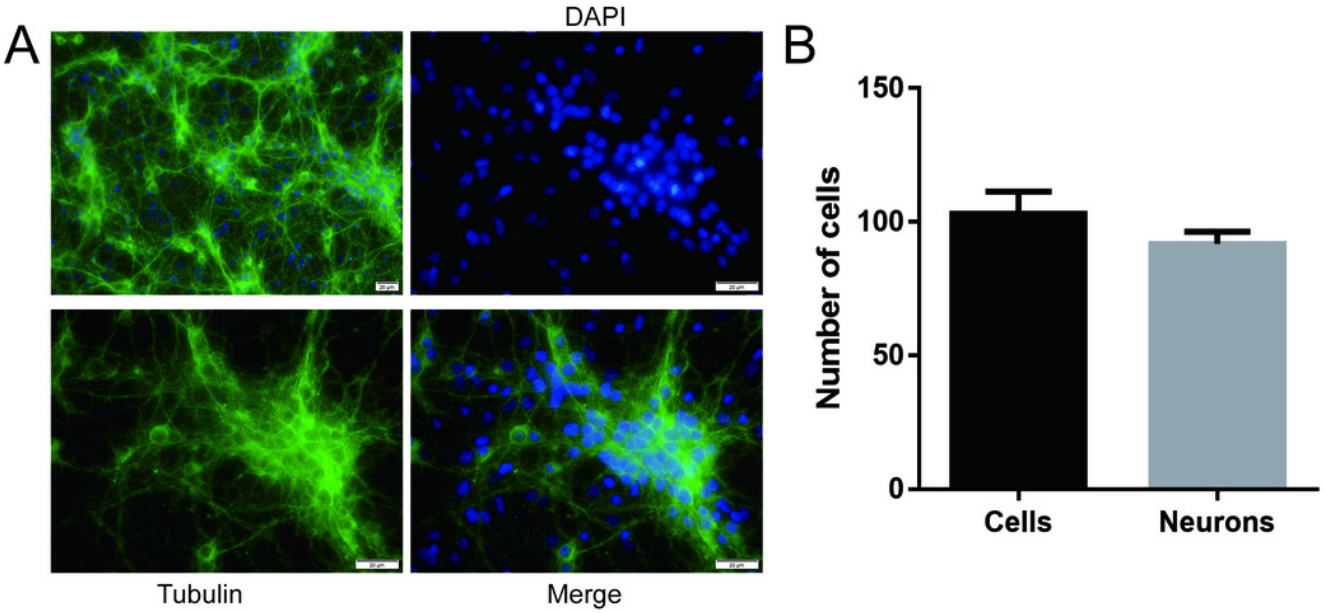

Figure 3

Fig. 3. Effects of DNLA on the cell viability of hippocampal neurons in SD Rat .

DNLA did not change significantly the cell vitality of hippocampal neurons (n = 3).

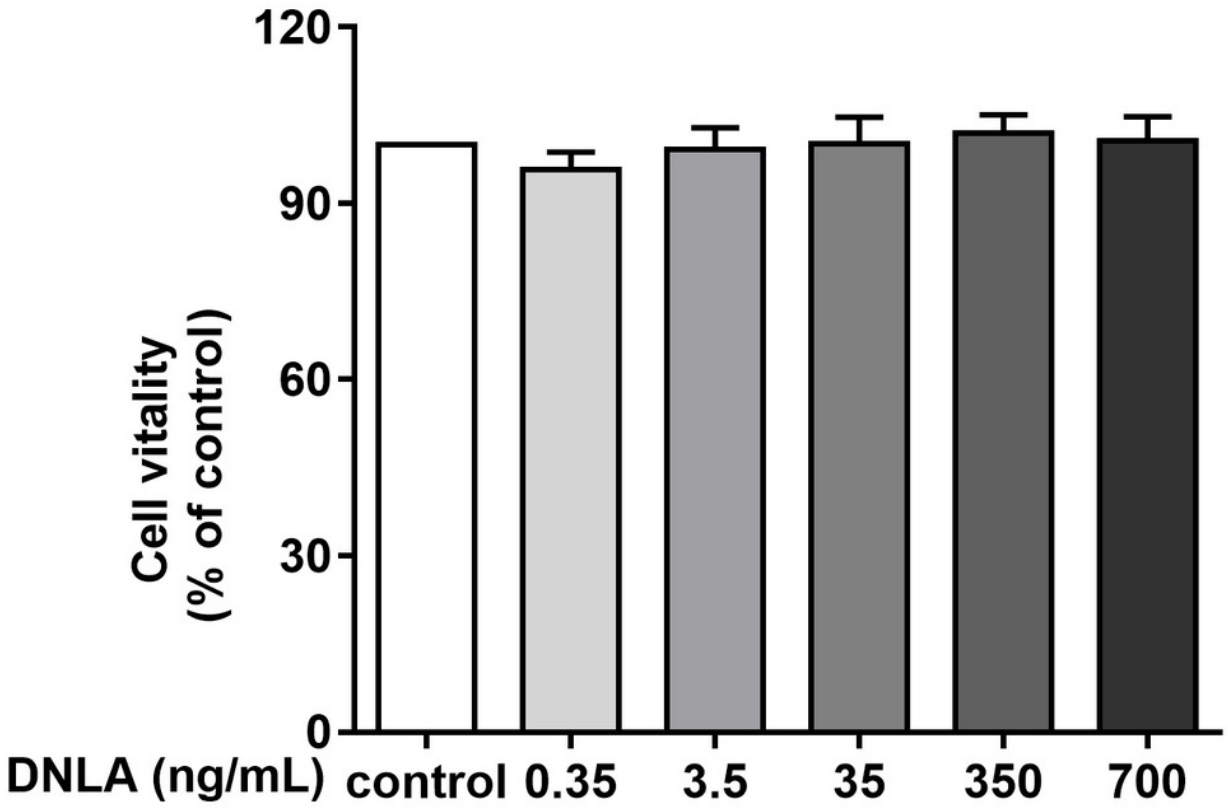

Figure 4

Fig. 4. DNLA reduced the production of Aβ through decreasing APP.

The protein expression level of $Aβ_{1-42}$(B), PS1(C) and APP(D) as determined from densitometric scans of Western blot. DNLA significantly decreased the protein expression of $Aβ_{1-42}$ (35,350,700 ng/mL) and APP (350 ng/mL). But did not change the protein expression of PS1. (A) is the representative strip of these protein. Data is presented as the mean ± SD (n = 4). *P < 0.05 versus the sham group.

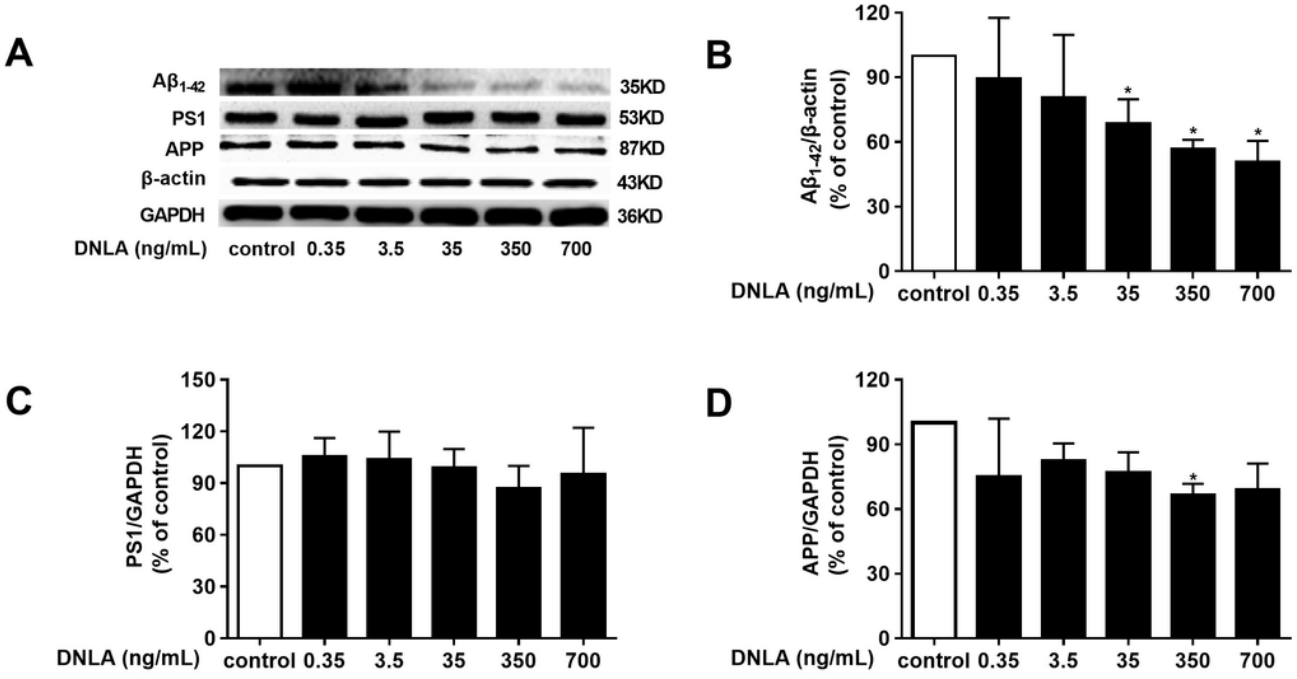

Figure 5

Fig. 5. DNLA reduced the production of Aβ through non-amyloidogenic pathways.

The protein expression level of ADAM17 (B) and ADAM10 (C) as determined from densitometric scans of Western blot. DNLA significantly decreased the protein expression of ADAM10 (35, 350, 700 ng/mL). However, it significantly increased the protein expression of ADAM17 (0.35 ng/mL). (A) is the representative strip of these protein. Data is presented as the mean ± SD (n = 4). * $P < 0.05$ versus the sham group.

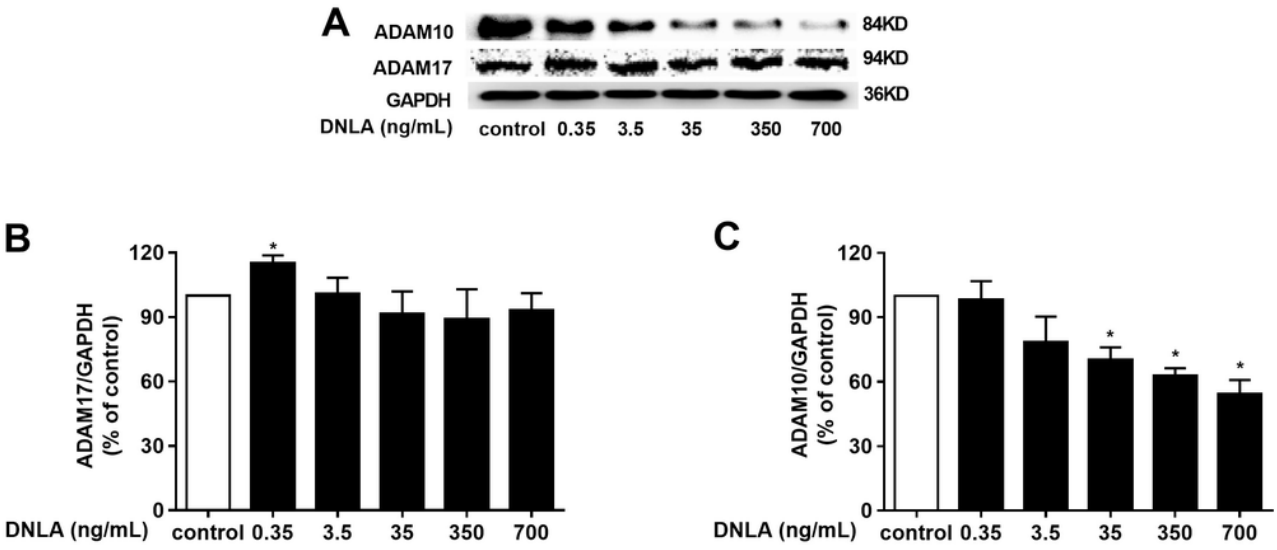

Figure 6

Fig. 6. DNLA reduced the production of Aβ through amyloidogenic pathways.

The protein expression level of BACE1 as determined from densitometric scans of Western blot. DNLA significantly decreased the protein expression of BACE1 (35,350,700 ng/mL). Data is presented as the mean ± SD (n = 4). * $P < 0.05$ versus the sham group.