Original paper

Folia Neuropathologica

Ciliary neurotrophic factor protects SH-SY5Y neuroblastoma cells against Aβ_{1-42}-induced neurotoxicity via activating the JAK2/STAT3 axis

Ke Wang[1], Minhao Xie[1], Ling Zhu[2], Xue Zhu[1], Kai Zhang[1], Fanfan Zhou[3]

[1]Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi 214063, Jiangsu Province, China, [2]Save Sight Institute, University of Sydney, NSW 2000, Australia, [3]Faculty of Pharmacy, University of Sydney, NSW 2006, Australia

Folia Neuropathol 2015; 53 (3): 226-235

DOI: 10.5114/fn.2015.54423

Abstract

The neurotoxicity of aggregated amyloid beta (Aβ) has been implicated as a critical cause in the pathogenesis of Alzheimer's disease (AD), which leads to neuronal cell damage by inducing oxidative stress and consequently triggering cell apoptosis. Recently, Aβ-dependent inactivation of the Janus tyrosine kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signaling pathway was found to play a critical role in the memory impairment related to AD. Previous research indicated that JAK2/STAT3 axis inactivation might be the result of aberrant reactive oxygen species (ROS) generation induced by Aβ in neurons. As the JAK2/STAT3 axis is a major transducer of ciliary neurotrophic factor (CNTF)-mediated neuroprotective activity, this study extensively evaluated whether activation of the JAK2/STAT3 axis by CNTF was responsible for the neuroprotective effect of this protein against Aβ_{1-42}-induced cytotoxicity, oxidative injury and cell apoptosis in human SH-SY5Y neuroblastoma cells. Our data showed that CNTF could attenuate or restore cell injury induced by Aβ_{1-42} in human SH-SY5Y neuroblastoma cells through activating the JAK2/STAT3 signaling pathway. Furthermore, CNTF strikingly prevented Aβ_{1-42}-induced mitochondrial dysfunction and activation of mitogen-activated protein kinases (MAPKs), an effect that could be potently attenuated by the specific JAK2 inhibitor AG490. In summary, this study confirmed the detailed mechanism accounting for CNTF's protective effect against Aβ_{1-42}-induced cytotoxic events in human SH-SY5Y neuroblastoma cells – information which might significantly contribute to better understanding of the mechanism of action of CNTF as well as providing a novel target in AD therapy.

***Key words:** Alzheimer's disease, Aβ1-42, ciliary neurotrophic factor, JAK2/STAT3.*

Introduction

Alzheimer's disease (AD) is the leading cause of dementia in aging adults, which currently has about 36 million cases worldwide [1,3,14]. Alzheimer's disease causes a large loss in brain weight and volume and affects some brain regions and neuronal populations [24,36]. Although the pathogenesis of AD

Communicating author:

Minhao Xie, Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi 214063, Jiangsu Province, China, phone: +86-510-85514482; fax: +86-510-85514482, e-mail: nypd0723@gmail.com

remains unclear, the key event appears to be the formation of a peptide known as amyloid beta (Aβ), with two major forms, $Aβ_{1-40}$ and $Aβ_{1-42}$ [2,16,26]. Amyloid β clusters into amyloid plaques on the blood vessels and the outer surface of neurons of the brain, leading to the killing of neurons [10]. The mechanism of Aβ-induced neurotoxicity is inconclusive. New evidence suggests that Aβ-dependent inactivation of the Janus tyrosine kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) axis in hippocampal neurons causes cholinergic dysfunction via pre- and post-synaptic mechanisms, which leads to memory impairment related to AD [7,8]. However, intracellular events accounting for the mechanism of this action in neurons remain elusive.

Oxidative stress is the redox state resulting from an imbalance between the generation and detoxification of reactive oxygen species (ROS) [33]. Increased cellular oxidative stress has been implicated in the pathogenesis of several neurodegenerative diseases, including Alzheimer's disease [27]. Aβ peptides are key stimuli of ROS generation, which could infuse into the brain through microdialysis probes and increase ROS production in an NMDA receptor- and nitric oxide-dependent manner [4]. A previous study indicated that aberrant activation of ROS generation might block activation of the JAK2/STAT3 axis in neurons, consequently disrupting the effect of neurotrophic factor and inducing neuronal damage in neurodegenerative diseases [19]. Thus, activation of the JAK2/STAT3 axis might induce a protective effect against oxidative stress induced by Aβ.

The major role of neurotrophic factors (NTFs) in synapse function along with evidence that synaptic failure is a critical early event in AD has put NTFs at the forefront of neuroprotective strategies for AD [32]. Ciliary neurotrophic factor (CNTF), a classic member of the NTFs, was first described as a growth factor that supports survival of chick ciliary ganglion neurons. Later, it was shown to be an important factor in the central and peripheral neurons of the nervous system [30]. CNTF binds to its α-receptor and two signal-transducing transmembrane subunits, LIFRβ and gp130, specifically activating the JAK2/STAT3 signaling pathway and thus preventing neuron death and facilitating axonal regeneration after nerve injury [4,28]. The neuroprotective effect of CNTF in AD has been previously reported; it might be mediated through modulating brain plasticity by promoting neurogenesis [12]. However, the precise cellular and molecular mechanisms underpinning such a neuroprotective effect remain far from clear.

In this study, we aimed to elucidate the role of the JAK2/STAT3 axis during the neuroprotective effect of CNTF against $Aβ_{1-42}$-induced oxidative injury in human SH-SY5Y neuroblastoma cells, which has been widely used as a typical model for AD [17,38]. Furthermore, we also elucidated the molecular events of both the upstream and downstream signaling pathways involved in this cellular process.

Material and methods

Materials and chemicals

Recombinant human CNTF was produced in *Escherichia coli* by our laboratory [37]. All cell culture reagents were purchased from Gibco (NY, USA). $Aβ_{1-42}$, AG490, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich (MO, USA). The Annexin V-FITC and PI double staining kit was obtained from BD Biosciences (CA, USA). Dimethyl sulfoxide (DMSO), RNase A, PVDF membranes and the enhanced chemiluminescence (ECL) detection kit were purchased from Beyotime (Nantong, China). Antibodies against Bcl-2, Bcl-xL and β-actin were obtained from Santa Cruz Biotechnology (CA, USA). Antibodies against JAK2, STAT3, phospho-JAK2, phospho-STAT3, JNK, ERK, p38, phospho-JNK, phospho-ERK and phospho-p38 were purchased from Abcam (MA, USA). Caspase-3 and caspase-9 fluorometric assay kits were obtained from BioVision (SF, USA). All other chemicals and reagents were of analytical grade.

Cell culture and treatment

The human neuroblastoma cell line (SH-SY5Y) was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). SH-SY5Y cells were cultured in flasks at 37°C under an atmosphere of 5% CO_2/95% air in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Before the experiments, $Aβ_{1-42}$ peptide was diluted to the desired concentrations and maintained for 3 days at 37°C for oligomerization. For the experiments, the cells were detached, re-seeded on plates and then incubated with or without drugs for the indicated time.

Cell growth assay

Cell proliferation was measured by the MTT assay as described before [22,43]. Briefly, 10 µl of MTT stock solution (5 mg/ml) was added to each well and incubated for 4 h at 37°C. The culture medium was then removed and 100 µl of DMSO was added to dissolve the formazan crystals. After mixing, absorbance was measured at 570 nm with an ELISA reader (Bio-Rad, CA, USA). Cell viability was also analyzed by the trypan blue exclusion assay. After treatment with the indicated drugs, cells were washed with phosphate buffer saline (PBS) twice at the end of the incubation period and trypsinized. The cells were re-suspended and subjected to trypan blue staining and then cell counting. Cell viability was expressed as a percentage value in relation to that of the control.

Cell apoptosis assay

Apoptosis of cells was examined by double staining with Annexin V-FITC and PI. After treatment, cells were washed twice with ice-cold PBS and re-suspended in 300 µl of binding buffer (Annexin V-FITC kit, Becton-Dickinson, CA, USA) containing 10 µl of Annexin V-FITC stock and 10 µl of PI. After 15 min incubation at room temperature in the dark, the samples were analyzed by flow cytometry. The Annexin V^+/PI^- cells were considered as apoptotic cells, the percentage of which was calculated by CellQuest software (Becton-Dickinson, CA, USA).

Reactive oxygen species detection

Reactive oxygen species (ROS) production was measured by flow cytometry using DCFH-DA staining. DCFH-DA is cleaved intracellularly by nonspecific esterases and transforms to highly fluorescent 2',7'-dichlorofluorescein (DCF) in the presence of ROS. Briefly, after treatment, the cells were incubated with DCFH-DA (20 µM) for 30 min at 37°C in the dark. After washing twice with PBS, the fluorescence intensity was measured by the microplate reader (Molecular Devices, CA, USA) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm. The level of ROS was expressed as a percentage value in relation to the control.

Western blot analysis

The treated cells were collected and lysed in ice-cold RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM ethylene diamine tetraacetic acid (EDTA), 20 mM NaF, 100 mM Na_3VO_4, 1% Nonidet P-40 (NP-40), 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mg/ml aprotinin and 10 mg/ml leupeptin) for 30 min. After centrifugation, protein concentration was determined by the Bradford method [21]. Cell lysates were separated by electrophoresis on 15% SDS-polyacrylamide gel (SDS-PAGE) and transferred onto PVDF membrane. After blocking with 5% bovine serum albumin (BSA) in the mixture of Tris-buffered saline and Tween-20 (TBST) for 1 h, membranes were incubated with primary antibody (diluted in 1 : 500) overnight and followed by incubation with secondary antibody (diluted in 1 : 1000) for 1 h at room temperature. Protein bands were visualized using the ECL assay kit (Beyotime, Nantong, China). The density of each band was normalized to the expression of β-actin.

Caspase activity assay

Caspase activity was assessed by fluorometric assay [38]. Cells were collected and lysed in caspase assay buffer containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 10 mM EGTA, 10 mM digitonin and 2 mM dithiothreitol (DTT). Protein was separated by centrifugation, the concentration of which was determined as previously described [21]. An equal amount of protein from each sample was incubated with caspase-3 substrate DEVD-AFC or caspase-9 substrate LEHD-AFC for 30 min at 37°C. The caspase activity was assessed by a spectrofluorometer (Molecular Devices, CA, USA) with an excitation wavelength of 400 nm and an emission wavelength of 505 nm.

Statistical analysis

Biostatistical analyses were conducted with the Prism 5.0 and SPSS 16.0 software packages. Data were represented as mean ± SEM. Comparisons between experimental and control groups were performed by one-way ANOVA and differences were considered to be statistically significant when *p* value was less than 0.05.

Results

CNTF induces JAK2/STAT3 activation in $A\beta_{1-42}$-treated SH-SY5Y cells

It is known that CNTF prevents neuronal cell death and facilitates axonal regeneration after nerve injury

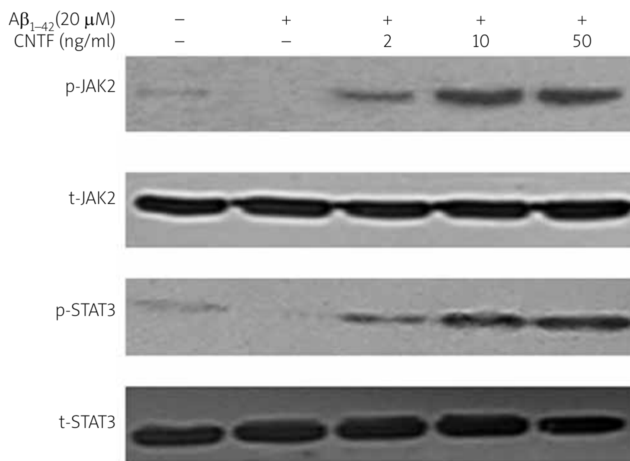

Fig. 1. The effect of CNTF on JAK2/STAT3 pathway activation in $A\beta_{1-42}$-treated SH-SY5Y cells. Cells were pretreated with or without $A\beta_{1-42}$ (20 μM) for 24 h and then exposed to the indicated concentrations of CNTF (2, 10, 50 ng/ml) for another 2 h. After treatment, the phosphorylation of JAK2 and STAT3 was evaluated by western blotting.

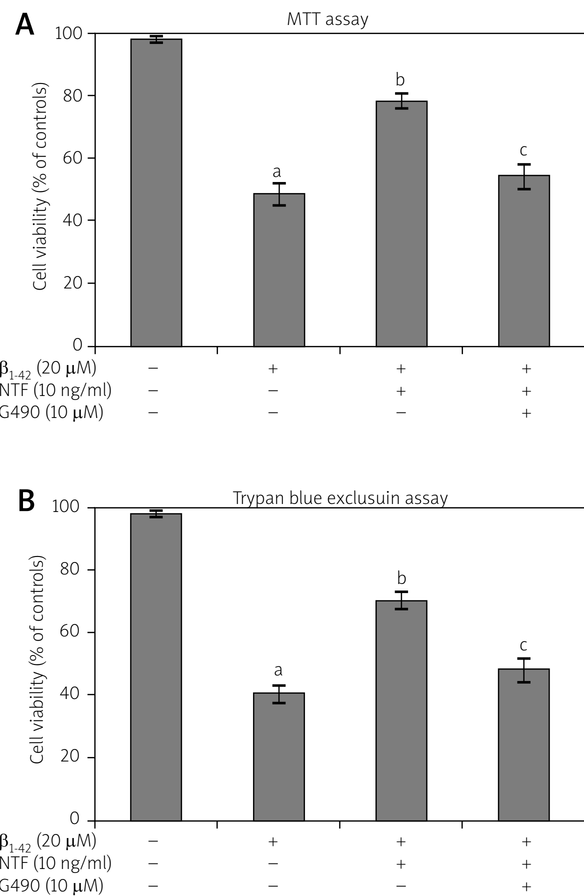

Fig. 2. The effect of CNTF on $A\beta_{1-42}$-induced cytotoxicity in SH-SY5Y cells. Cells were treated with $A\beta_{1-42}$ (20 μM) in the absence or presence of CNTF (10 ng/ml) and AG490 (10 μM) for 24 h. The viability of SH-SY5Y cells was assessed by MTT assay **(A)** and trypan blue exclusion assay **(B)**. Results are shown as mean ± SEM of three experiments with each including triplicate sets. a: $p < 0.05$ vs. control, b: $p < 0.05$ vs. $A\beta_{1-42}$, c: $p < 0.05$ vs. $A\beta_{1-42}$ + CNTF.

directly via activating the JAK2/STAT3 signaling pathway [28]. To test whether JAK2/STAT3 pathway was activated by CNTF in $A\beta_{1-42}$-treated human SH-SY5Y neuroblastoma cells, cells were pre-incubated with $A\beta_{1-42}$ (20 μM) for 24 h and then exposed to the indicated concentrations of CNTF (0, 2, 10, 50 ng/ml) for another 2 h. As shown in Fig. 1, treatment with $A\beta_{1-42}$ alone could inactivate the JAK2/STAT3 pathway, whereas co-treatment with CNTF significantly stimulated the phosphorylation of JAK2 and STAT3 in a dose-dependent manner. Our data indicated that CNTF potently induced the activation of the JAK2/STAT3 pathway in $A\beta_{1-42}$-treated human SH-SY5Y neuroblastoma cells.

CNTF protects SH-SY5Y cells from $A\beta_{1-42}$-induced cytotoxicity

$A\beta_{1-42}$ impairing neuronal cells has been considered as one of the major causes of AD [31]; therefore, in this study, $A\beta_{1-42}$ was employed as a neurotoxicant. The neuroprotective effect of CNTF against $A\beta_{1-42}$-induced cytotoxicity was evaluated as the viability of SH-SY5Y cells using the MTT assay and trypan blue exclusion assay. As shown in Fig. 2, $A\beta_{1-42}$ (20 μM) exhibited a remarkable inhibitory effect on the growth of SH-SY5Y cells. However, this cytotoxic effect was attenuated by co-treatment with CNTF (10 ng/ml). To investigate the involvement of JAK2/STAT3 signaling in the protective effect of CNTF against $A\beta_{1-42}$ induced cytotoxicity, we assessed cell viability with the co-treatment of the JAK2 inhibitor AG490 in the presence of both CNTF and $A\beta_{1-42}$. Our results indicated that the suppressive effect of CNTF on $A\beta_{1-42}$-induced cytotoxicity was significantly diminished in the presence of AG490 in human SH-SY5Y neuroblastoma cells.

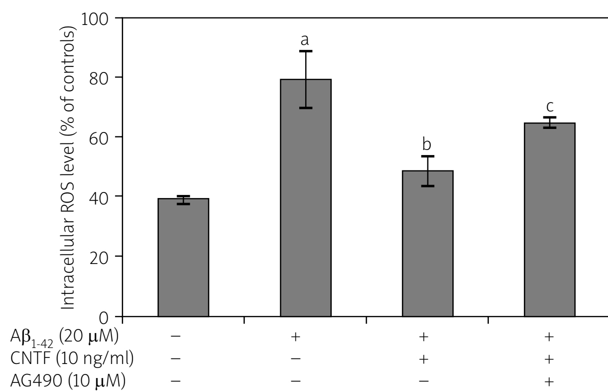

Fig. 3. The effect of CNTF on $A\beta_{1-42}$-induced oxidative stress in SH-SY5Y cells. Cells were treated with $A\beta_{1-42}$ (20 μM) in the absence or presence of CNTF (10 ng/ml) and AG490 (10 μM) for 6 h. Oxidative stress was assessed as the intracellular ROS level. Results are shown as mean ± SEM of three experiments with each including triplicate sets. a: $p < 0.05$ vs. control, b: $p < 0.05$ vs. $A\beta_{1-42}$, c: $p < 0.05$ vs. $A\beta_{1-42}$ + CNTF.

CNTF protects SH-SY5Y cells from $A\beta_{1-42}$-induced oxidative injury

$A\beta_{1-42}$-induced oxidative stress was considered as crucial to the pathophysiology of AD [6]. In this study, oxidative stress was assessed by measuring the intracellular ROS level using the ROS-sensitive fluorescence probe DCF. As shown in Fig. 3, after exposure to $A\beta_{1-42}$ (20 μM) for 6 h, the intracellular ROS level was significantly increased (198.5% of the control), which suggested induced oxidative stress in SH-SY5Y cells. After co-treatment with CNTF (10 ng/ml), the intracellular ROS level was significantly decreased compared to that of $A\beta_{1-42}$ treatment alone (122.3% of the control). In addition, AG490 inhibited the protective effect of CNTF on $A\beta_{1-42}$-induced oxidative injury in SH-SY5Y cells. Our results suggested that CNTF could prevent $A\beta_{1-42}$-induced oxidative injury in human SH-SY5Y neuroblastoma cells, possibly via activating the JAK2/STAT3 signaling pathway.

CNTF protects SH-SY5Y cells from $A\beta_{1-42}$-induced apoptosis

$A\beta_{1-42}$-induced neuronal apoptosis in the brain and primary neuronal culture might be responsible in part for the cognitive decline found in AD [16]. Apoptosis of SH-SY5Y cells with or without treatment was evaluated by dual-staining with Annexin V-FITC/PI. As shown in Fig. 4, treatment with $A\beta_{1-42}$ (20 μM) remarkably increased the percentage of early apoptotic cells, while co-treatment with CNTF (10 ng/ml) resulted in decreased cell apoptosis from 38.67 ± 6.43% to 11.28 ± 2.62%. The addition of AG490 reversed the protective effect of CNTF, increasing the percentage of apoptosis to 24.77 ± 4.15%. These data suggest that CNTF could prevent $A\beta_{1-42}$-induced cell apoptosis in human SH-SY5Y neuroblastoma cells, an effect which was also closely connected with activation of the JAK2/STAT3 pathway.

CNTF inhibits $A\beta_{1-42}$-induced mitochondrial dysfunction

Mitochondrial dysfunction is an early event of cell apoptosis [39]. Increasing evidence indicates that Aβ induces oxidative injury and neuronal apoptosis through mediating mitochondrial dysfunction [25]. To investigate the effect of CNTF on $A\beta_{1-42}$-induced mitochondrial dysfunction, the expression of anti-apoptotic members (Bcl-xL and Bcl-2) and activity of caspases were assessed, these genes being downstream targets of the JAK2/STAT3 pathway. As shown in Fig. 5, $A\beta_{1-42}$ (20 μM) treatment significantly down-regulated the expression of Bcl-xL and Bcl-2 and up-regulated the activity of initiator caspase-9 and effector caspase-3. However, CNTF (10 ng/ml) co-treatment substantially reversed the regulatory effect of $A\beta_{1-42}$. In addition, AG490 attenuated the protective effect of CNTF on $A\beta_{1-42}$-induced mitochondrial dysfunction in human SH-SY5Y neuroblastoma cells.

CNTF inhibits $A\beta_{1-42}$-induced JNK and ERK activation

Accumulating evidence has suggested that the MAPK signaling pathway plays an important role in neuronal death in AD [42]. $A\beta_{1-42}$-induced oxidative stress influences the decision of susceptible neurons to undergo either apoptosis or proliferation, a process which is likely mediated through the MAPK signaling pathway [34]. In this study, the phosphorylation of JNK, ERK and p38, the most extensively studied vertebrate MAPKs, was assessed by western blot analysis. As shown in Fig. 6, $A\beta_{1-42}$ (20 μM) treatment significantly induced the phosphorylation of JNK and ERK but not p38 (data not shown), an effect that was inhibited by CNTF (10 ng/ml). Again, the

A

Control

1.56%

$A\beta_{1-42}$ (20 μM)

38.23%

$A\beta_{1-42}$ (20 μM) + CNTF (10 ng/ml)

10.13%

$A\beta_{1-42}$ (20 μM) + CNTF (10 ng/ml) + AG490 (10 μM)

22.38%

Propidium ioide

Annexin V-FITC

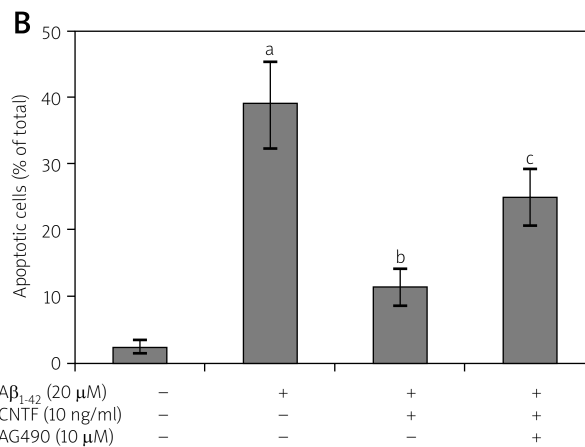

Fig. 4. The effect of CNTF on $A\beta_{1-42}$-induced apoptosis in SH-SY5Y cells. Cells were treated with $A\beta_{1-42}$ (20 μM) in the absence or presence of CNTF (10 ng/ml) and AG490 (10 μM) for 24 h. Cell apoptosis was evaluated by flow cytometry. **(A)** Flow cytometry analysis of cell apoptosis using Annexin V-FITC/PI dual staining. **(B)** Percentage distribution of apoptotic cells. Results are shown as mean ± SEM of three experiments with each including triplicate sets. a: $p < 0.05$ vs. control, b: $p < 0.05$ vs. $A\beta_{1-42}$, c: $p < 0.05$ vs. $A\beta_{1-42}$ + CNTF.

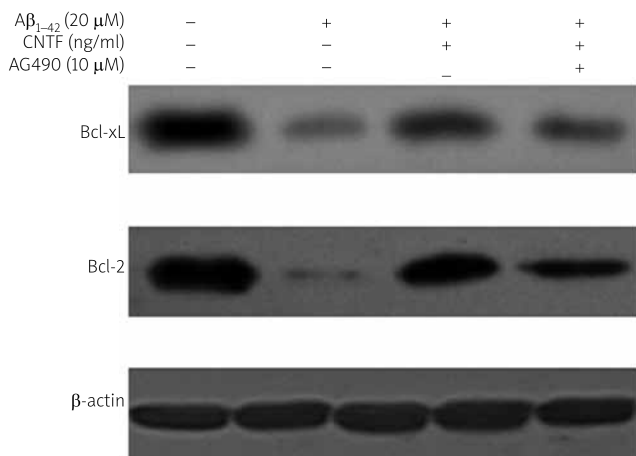

Fig. 5. The effect of CNTF on $A\beta_{1-42}$-induced mitochondrial dysfunction in SH-SY5Y cells. Cells were treated with $A\beta_{1-42}$ (20 μM) in the absence or presence of CNTF (10 ng/ml) and AG490 (10 μM) for 24 h. **(A)** Expression of Bcl-xL and Bcl-2 was assessed by western blotting. **(B)** Activity of caspase-3 and -9 was determined by fluorometric assay. Results are shown as mean ± SEM of three experiments with each including triplicate sets. a: $p < 0.05$ vs. control, b: $p < 0.05$ vs. $A\beta_{1-42}$, c: $p < 0.05$ vs. $A\beta_{1-42}$ + CNTF.

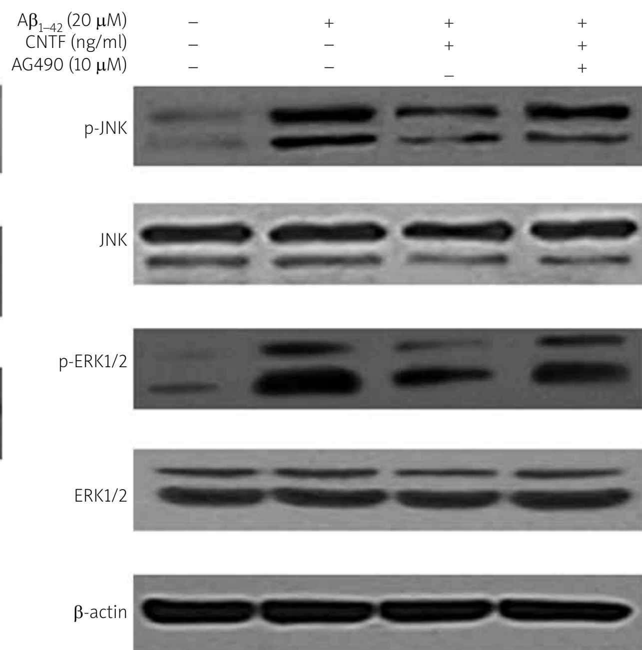

Fig. 6. The effect of CNTF on $A\beta_{1-42}$-induced JNK and ERK activation in SH-SY5Y cells. Cells were treated with $A\beta_{1-42}$ (20 μM) in the absence or presence of CNTF (10 ng/ml) and AG490 (10 μM) for 6 h. Expression of JNK and ERK was assessed by western blotting.

effect of CNTF on $A\beta_{1-42}$-induced JNK and ERK activation was abolished by co-treatment with AG490 in human SH-SY5Y neuroblastoma cells.

Discussion

Alzheimer's disease is the most common neurodegenerative dementia in the elderly, affecting cognition, behavior and functioning due to neuron loss [35]. Neurodegeneration possibly results from the abnormal accumulation of extracellular Aβ; therefore, Aβ is a promising therapeutic target of AD [20]. CNTF is a pleiotropic cytokine with neurotrophic properties for a number of neurons *in vitro* and *in vivo*. It is also one of the most active neurotrophic factors widely studied in promoting neurogenesis [15,23]. Previous studies reported that treatment of CNTF in two AD mice models prevented Aβ oligomer-induced neuronal damage and neurobehavioral impairments [29]; however, the molecular mechanism underpinning this effect was largely unknown. Consistently, we confirmed the protective effect of CNTF on $A\beta_{1-42}$-induced cytotoxicity in human SH-SY5Y neuroblastoma cells in the current study. Furthermore, we elucidated the involvement of the critical signaling pathway in the molecular response of CNTF.

CNTF belongs to the IL-6 family of cytokines, signaling of which is mainly mediated through activation of the receptor-associated JAK2/STAT3 pathway [18]. Recent studies have demonstrated that the JAK2/STAT3 pathway is involved in multiple physiological processes of the nervous system [11, 41]. Chiba *et al.* reported that Aβ-dependent inactivation of the JAK2/STAT3 axis could lead to memory loss through cholinergic dysfunction [9]. In the current study, our results demonstrated that the JAK2/STAT3 pathway was inactivated upon the treatment with $A\beta_{1-42}$ in human SH-SY5Y neuroblastoma cells, whereas the further co-treatment with CNTF significantly stimulated the phosphorylation of JAK2 and STAT3 in a dose-dependent manner and protected cells against $A\beta_{1-42}$-induced cytotoxicity. Additionally, the JAK2 inhibitor AG490 largely attenuated the protective effect of CNTF on $A\beta_{1-42}$-induced toxicity in human SH-SY5Y neuroblastoma cells. Our study

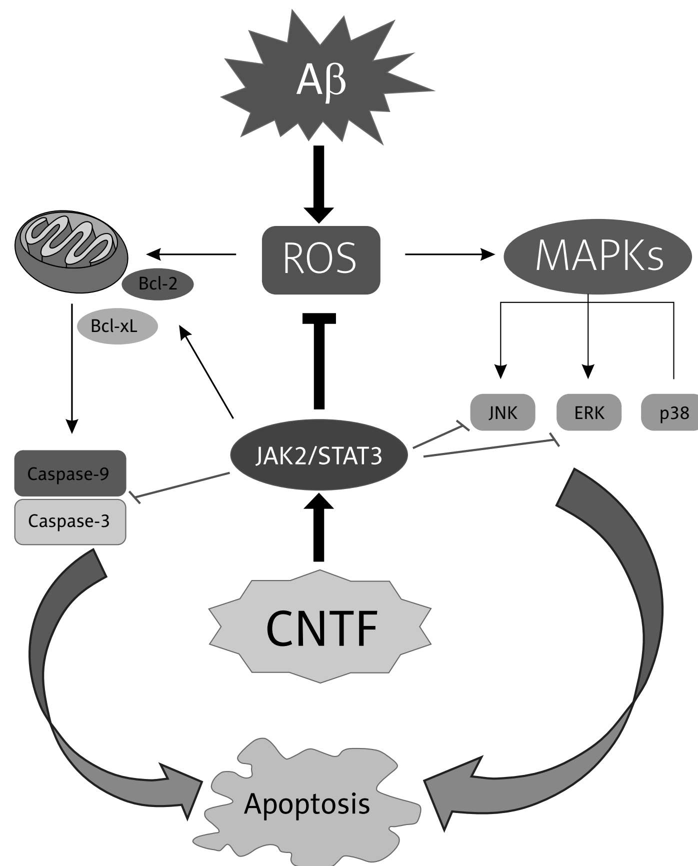

Fig. 7. The proposed model of CNTF neuroprotective effect against $A\beta_{1-42}$-induced cytotoxicity in human SH-SY5Y neuroblastoma cells.

suggested that the JAK2/STAT3 pathway might be the major transducer of CNTF-mediated neuroprotective activity.

Oxidative stress is one of the early events in AD, and is also implicated as an important mediator of the onset, progression and pathogenesis of AD. Several studies have suggested that oxidative stress plays a key role in Aβ-mediated neuronal cytotoxicity by triggering or facilitating neurodegeneration through a wide range of molecular events that eventually lead to neuronal cell loss [6]. In our study, after exposure of SH-SY5Y cells to $A\beta_{1-42}$ for 6 h, the intracellular ROS level was significantly increased; however, co-treatment with CNTF potently inhibited this effect, suggesting that CNTF could prevent $A\beta_{1-42}$-induced oxidative injury in SH-SY5Y cells. Recent studies demonstrated that the JAK2/STAT3 pathway was involved in regulating oxidative stress in certain types of neurons. We also confirmed that activation of the JAK2/STAT3 pathway was involved in the protective effect of CNTF against $A\beta_{1-42}$-induced oxidative injury.

To further investigate the molecular mechanisms underlying this neuroprotective effect, the ROS-related downstream signaling pathways including the mitochondrial pathway and MAPK cascades were monitored. Zhao *et al.* showed that the JAK2/STAT3 pathway could modulate the expression of anti-apoptotic members (Bcl-xL and Bcl-2) and activity of caspases [40]. Our present study also proved that activation of the JAK2/STAT3 pathway could reverse the decreased expression of Bcl-xL and Bcl-2 as well as activation of caspases induced by $A\beta_{1-42}$ in human SH-SY5Y neuroblastoma cells. Oxidative stress is one of the major stimuli of MAPK cascades, pathways widely involved in apoptotic signal transduction. Ghribi *et al.* reported that administration of $A\beta_{1-42}$ into rabbit brain induced apoptosis accompanied with activation of JNK and ERK, but not p38 [13]. Our study showed that treatment with $A\beta_{1-42}$ significantly induced the phos-

phorylation of JNK and ERK in human SH-SY5Y neuroblastoma cells, an effect that could be abolished by co-treatment with CNTF. JNK and ERK pathways are also targets of the JAK2/STAT3 axis, and co-treatment with AG490 inhibited the effect of CNTF on $A\beta_{1-42}$-induced JNK and ERK activation. Our data suggested that CNTF could inhibit $A\beta_{1-42}$-induced mitochondrial dysfunction and MAPK activation via activating the JAK2/STAT3 pathway in human SH-SY5Y neuroblastoma cells.

In conclusion, our study extensively evaluated the protective effect of CNTF against $A\beta_{1-42}$-induced neurotoxicity in human SH-SY5Y neuroblastoma cells. More importantly, we provided evidence that such a neuroprotective effect of CNTF was largely mediated through activation of the JAK2/STAT3 signaling pathway (Fig. 7). Our findings might significantly contribute to better understanding of the mechanism of action of CNTF and constitute a basis for future development of neuronal growth factors as potential drugs for the treatment of AD.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation (81300787), the Natural Science Foundation of Jiangsu Province (BK2011168, BK2012105, BK20141103) and the Major Project of Wuxi Municipal Health Bureau (ZS201401).

Disclosure

Authors report no conflict of interest.

References

1. Alzheimer's Association. Alzheimer's Association report. 2013 Alzheimer's disease facts and figures. Alzheimers Dement 2013; 9: 208-245.
2. Armstrong RA. What causes alzheimer's disease? Folia Neuropathol 2013; 51: 169-188.
3. Armstrong RA. A critical analysis of the 'amyloid cascade hypothesis'. Folia Neuropathol 2014; 52: 211-225.
4. Bonni A, Frank DA, Schindler C, Greenberg ME. Characterization of a pathway for ciliary neurotrophic factor signaling to the nucleus. Science 1993; 262: 1575-1579.
5. Butterfield AD. Amyloid β-peptide (1-42)-induced Oxidative Stress and Neurotoxicity: Implications for Neurodegeneration in Alzheimer's Disease Brain. A Review. Free Radic Res 2002; 36: 1307-1313.
6. Butterfield DA, Swomley AM, Sultana R. Amyloid β-peptide (1-42)-induced oxidative stress in Alzheimer disease: Importance in disease pathogenesis and progression. Antioxid Redox Signal 2013; 19: 823-835.
7. Chiba T, Yamada M, Aiso S. Targeting the JAK2/STAT3 axis in Alzheimer's disease. Expert Opin Ther Targets 2009; 10: 1155-1167
8. Chiba T, Yamada M, Sasabe J, Terashita K, Shimoda M, Matsuoka M, Aiso S. Amyloid-β causes memory impairment by disturbing the JAK2/STAT3 axis in hippocampal neurons. Mol Psychiatry 2008; 14: 206-222.
9. Chiba T, Yamada M, Sasabe J, Terashita K, Shimoda M, Matsuoka M, Aiso S. Amyloid-β causes memory impairment by disturbing the JAK2/STAT3 axis in hippocampal neurons. Mol Psychiatry 2009; 14: 206-222.
10. Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. Hum Mol Genet 2010; 19: R12-R20.
11. Dominguez E, Rivat C, Pommier B, Mauborgne A, Pohl M. JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat. J Neurochem 2008; 107: 50-60.
12. Garcia P, Youssef I, Utvik JK, Florent-Béchard S, Barthélémy V, Malaplate-Armand C, Kriem B, Stenger C, Koziel V, Olivier J-L. Ciliary neurotrophic factor cell-based delivery prevents synaptic impairment and improves memory in mouse models of Alzheimer's disease.J Neurosci 2010; 30: 7516-7527.
13. Ghribi O, Prammonjago P, Herman MM, Spaulding NK, Savory J. Aβ (1–42)-induced JNK and ERK activation in rabbit hippocampus is differentially regulated by lithium but is not involved in the phosphorylation of tau. Mol Brain Res 2003; 119: 201-206.
14. Hampel H, Prvulovic D, Teipel S, Jessen F, Luckhaus C, Frölich L, Riepe MW, Dodel R, Leyhe T, Bertram L. The future of Alzheimer's disease: the next 10 years. Prog Neurobiol 2011; 95: 718-728.
15. Ip N, Li Y, Van de Stadt I, Panayotatos N, Alderson R, Lindsay R. Ciliary neurotrophic factor enhances neuronal survival in embryonic rat hippocampal cultures. J Neurosci 1991; 11: 3124-3134.
16. Ittner LM, Götz J. Amyloid-β and tau – a toxic pas de deux in Alzheimer's disease. Nat Rev Neurosci 2011; 12: 67-72.
17. Jämsä A, Hasslund K, Cowburn RF, Bäckström A, Vasänge M. The retinoic acid and brain-derived neurotrophic factor differentiated SH-SY5Y cell line as a model for Alzheimer's disease-like tau phosphorylation. Biochem Biophys Res Commun 2004; 319: 993-1000.
18. Kaur N, Kim IJ, Higgins D, Halvorsen SW. Induction of an interferon-gamma Stat3 response in nerve cells by pre-treatment with gp130 cytokines. J Neurochem 2003; 87: 437-447.
19. Kaur N, Lu B, Ward S, Halvorsen S. Inducers of oxidative stress block ciliary neurotrophic factor activation of Jak/STAT signaling in neurons. J Neurochem 2005; 92: 1521-1530.
20. Klein WL, Krafft GA, Finch CE. Targeting small Aβ oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci 2001; 24: 219-224.
21. Kruger NJ. The Bradford method for protein quantitation. Methods Mol Biol 1994; 32: 9-15.
22. Li J, Ji X, Zhang J, Shi G, Zhu X, Wang K. Paeoniflorin attenuates Abeta25-35-induced neurotoxicity in PC12 cells by preventing mitochondrial dysfunction. Folia Neuropathol 2014; 52: 285-290.

23. Li P, Wang Z, Yan J, Li Z, Jiang C, Ni X, Yang Y, Liu F, Lu C. Neuro-protective effects of CNTF on hippocampal neurons via an unknown signal transduction pathway. Chin Sci Bull 2006; 51: 48-53.
24. Mavroudis IA, Manani MG, Petrides F, Petsoglou C, Njau SN, Costa VG, Baloyannis SJ. Dendritic and spinal alterations of neurons from Edinger-Westphal nucleus in Alzheimer's disease. Folia Neuropathol 2014; 52: 197-204.
25. Onyango IG, Khan SM. Oxidative Stress, Mitochondrial Dysfunction, and Stress Signaling in Alzheimers Disease. Curr Alzheimer Res 2006; 3: 339-349.
26. Palop JJ, Mucke L. Amyloid-[beta]-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci 2010; 13: 812-818.
27. Perry G, Cash AD, Smith MA. Alzheimer disease and oxidative stress. J BioMed Biotechnol 2002; 2: 120-123.
28. Peterson WM, Wang Q, Tzekova R, Wiegand SJ. Ciliary neurotrophic factor and stress stimuli activate the Jak-STAT pathway in retinal neurons and glia. J Neurosci 2000; 20: 4081-4090.
29. Qu HY, Zhang T, Li XL, Zhou JP, Zhao BQ, Li Q, Sun MJ. Transducible P11-CNTF rescues the learning and memory impairments induced by amyloid-beta peptide in mice. Eur J Pharmacol 2008; 594: 93-100.
30. Richardson P. Ciliary neurotrophic factor: a review. Pharmacol Ther 1994; 63: 187-198.
31. Roher AE, Lowenson JD, Clarke S, Woods AS, Cotter RJ, Gowing E, Ball MJ. Beta-amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. Proc Nat Acad Sci USA 1993; 90: 10836-10840.
32. Schindowski K, Belarbi K, Buee L. Neurotrophic factors in Alzheimer's disease: role of axonal transport. Genes Brain Behav 2008; 7: 43-56.
33. Sies H. Oxidative stress. Academic Press, London 1985.
34. Son Y, Cheong Y-K, Kim N-H, Chung H-T, Kang DG, Pae H-O. Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? J Signal Transduct 2011; 2011:792639.
35. Sonkusare S, Kaul C, Ramarao P. Dementia of Alzheimer's disease and other neurodegenerative disorders – memantine, a new hope. Pharmacol Res 2005; 51: 1-17.
36. Sperling RA, Dickerson BC, Pihlajamaki M, Vannini P, LaViolette PS, Vitolo OV, Hedden T, Becker JA, Rentz DM, Selkoe DJ. Functional alterations in memory networks in early Alzheimer's disease. Neuromolecular Med 2010; 12: 27-43.
37. Wang K, Zhou F, Zhu L, Zhu X, Zhang K, Zhu L. High level soluble expression, purification, and characterization of human ciliary neuronotrophic factor in Escherichia coli by single protein production system. Protein Expr Purif 2014; 96: 8-13.
38. Wang K, Zhu L, Zhu X, Zhang K, Huang B, Zhang J, Zhang Y, Zhu L, Zhou B, Zhou F. Protective Effect of Paeoniflorin on Aβ25–35-Induced SH-SY5Y Cell Injury by Preventing Mitochondrial Dysfunction. Cell Mol Neurobiol 2014; 34: 227-234.
39. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev 2001; 15:2922-2933.
40. Zhao J, Li G, Zhang Y, Su X, Hang C. The potential role of JAK2/STAT3 pathway on the anti-apoptotic effect of recombinant human erythropoietin (rhEPO) after experimental traumatic brain injury of rats. Cytokine 2011; 56: 343-350.
41. Zhong Z, Wen Z, Darnell J. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994; 264: 95-98.
42. Zhu X, Lee H-G, Raina AK, Perry G, Smith MA. The role of mitogen-activated protein kinase pathways in Alzheimer's disease. Neurosignals 2002; 11: 270-281.
43. Zhu X, Wang K, Zhang K, Zhu L, Zhou F. Ziyuglycoside II induces cell cycle arrest and apoptosis through activation of ROS/JNK pathway in human breast cancer cells. Toxicol Lett 2014; 227: 65-73.