International Journal of Medicinal Plants and Natural Products (IJMPNP)
Volume 5, Issue 1, 2019, PP 8-15
ISSN 2454-7999 (Online)
DOI: http://dx.doi.org/10.20431/2454-7999.0501002
www.arcjournals.org

Effect of SBF on Aβ25-35 Induced- Abnormal Changes of PKA and tau Protein Phosphorylation in N2a Cells

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Abstract:

Objective:

To investigate the effects of Scutellariabarbata flavonoids (SBF) on abnormal changes of N2a cell protein kinase (PKA) and Tau protein phosphorylation induced by β-amyloid25-35 (Aβ25-35). Methods: N2a cells were cultured and randomly divided into 7 groups, including control group, PKA inhibitor H-89 group, model group, Aβ25-35+H-89 group and SBF three dose treatment groups. The control group was not treated. H-89 with a final concentration of 48 nmol /L was added to the H-89 group, and fully act for 12 h. Aβ25-35 of 40 μmol/L was added to the model group, and work for 12 h. SBF of 1.125 mg/L, 2.25 mg/L and 4.5 mg/L were added to SBF dose treatment groups respectively, and H-89 of 48 nmol /L was added at the same time, after acting cells for 12 h, Aβ25-35 with a final concentration of 40 μmol/L was added to continue the action for 12 h. Western blot was used to detect the phosphorylation expression levels of PKA and Tau protein at Ser199, Ser214, Ser404 and Thr231 of N2a cells to each group. Results: Compared with the control group, the protein expression levels of PKA, p-Tau (Ser199), p-Tau (Ser214), p-Tau (Ser404) and p-Tau (Thr231) were significantly increased in the model group ($p<0.01$).In the H-89 group, except for the decreased protein expression levels of PKA, the phosphorylated Tau protein expression levels at Ser199, Ser214, Ser404 and Thr231 of four sites were increased($p<0.01$). Compared with the model group, protein expression levels of PKA, p-Tau (Ser199) and p-Tau (Ser404) in Aβ25-35+H-89 group were significantly decreased ($P <0.01$), as well as the phosphorylated Tau expression levels at Ser214 and Thr231 sites ($P <0.05$). Compared with group Aβ25-35+H-89, the protein expression levels of p-Tau (Ser404), p-Tau (Ser199), p-Tau (Ser214) and p-Tau (Thr231) were all decreased in the SBF lowdose treatment group, but the protein expression levels of p-Tau (Ser404) were increased. In the SBF medium dose treatment group, the protein expression levels of PKAand p-Tau (Thr231) were increased, while the phosphorylated Tau protein expression levels at Ser199, Ser404 and Ser214 sites were decreased. The expression levels of phosphorylated Tau protein at Ser214 and Ser404 sites were decreased in the SBF highdose group, while the protein express levels of PKA, p-Tau (Ser199) and p-Tau (Thr231) were increased.

Conclusion:

SBF can inhibit the hyperphosphorylated Tau protein expression levels at Ser199, Ser214, Ser404, and Thr231 sites by regulating PKA activity.

Keywords: *Scutellariabarbata flavonoids; PKA; Tau protein phosphorylation; N2a cells*

1. INTRODUCTION

Alzheimer's disease (AD), also known as senile dementia, is a neurodegenerative disease that occurs frequently with age. Its clinical manifestations are mainly progressive memory impairment, cognitive dysfunction, personality changes and language disorders, which frequently occur in the elderly [1]. The most important pathological feature of AD is the formation of extracellular amyloid polypeptide deposition known as senile plaques (SP) and intracellular neurofibrillary tangles (NFT) [2]. There is no effective strategy for treatment of AD at present in clinic, then, the early prevention and treatment of

AD is particularly important. Studies in AD model animals have found that it is feasible to reduce the formation of NFT in the brain by reducing the content of intracellular hyperphosphorylated Tau protein, which is closely related to improving the cognitive ability of AD patients [3]. More and more cell and tissue research results support the effectiveness of a series of traditional Chinese medicine compounds or extracts to target Tau in the treatment of AD. SBF was isolated extracts from the arial parts of *Scutellariabarbata D. Don,* and related studies have reported that SBF have a stronger improvement effect on memory impairment and nerve damage in animals with simulated AD, and have an inhibitory effect on Tau protein hyperphosphorylation[4]. In vitro studies have also showed that they can protect astrocytes from damage caused by $A\beta_{25-35}$, and can inhibit abnormal expression of GSK3βand Tau protein hyperphosphorylation which induced by $A\beta_{25-35}$[5-6]. However, it has not been reported whether SBF can regulate Tau protein hyperphosphorylation by affecting PKA. In the present study, PKA inhibitor H-89 was used to investigate whether SBF regulates the Tau protein phosphorylation at Ser199, Ser214, Ser404 and Thr231 sites by affecting PKA, so as to further clarify the mechanism of SBF anti-AD.

2. Materials and Methods

2.1. Experimental Material

Scutellariabarbata flavonoids (SBF) were prepared by the Institute of Traditional Chinese Medicine of Chengde Medical College, and their total flavonoids purity was 93.1%. N2a cells is mouse brain neuroma cells and provided by Beijing Dingguo Changsheng Biotechnology Co. Ltd., China. High sugar DMEM (Catalog No.8511010130) was purchased from GENVIEW Company, China. Fetal bovine serum (Catalog No. SF0016500)was supplied by France Scitecher Company. $A\beta_{25-35}$(Catalog No. A0331A)provided by Dalian Meilun Company, China. PKA inhibitor H-89 (Catalog No. Hy-15979A)was purchased from MCE Company, China. p-Tau (Ser199)primary antibody was supplied by abcam Company, British. p-Tau (Ser214),p-Tau (Ser404) andp-Tau (Thr231) primary antibody were supplied by American SAB Company. PKA (Catalog No. cc36131) primary antibody was bought from American Bioworld Company. And goat anti-rabbit secondary antibody (Catalog No. GR288027-15) was supplied by abcam Company, British. β-actinprimary antibody (Catalog No. M1210-2) was purchased from Hua'an Biotechnology Co., Ltd.β-actinsecondary antibody (Catalog No. AS100) was supplied by American Medicine Mingkangde Company. Other reagents were from ordinary commercial sources.

3. Experimental Methods

3.1. N2a Cell Culture

N2a cells were cultured in high glucose DMEM complete culture medium, which contains 10% FBS and 100 U/mL penicillin and placed in 37 ℃ and 5% CO2 incubator. The cells were adhered growth and the culture medium was replaced in each day. The cells were performed to passage when the cells density reaches the confluence degree of 80% - 90%. The cells were digested 20 s with 0.25% trypsin and then dried digestion for 3 - 5 min. After the cells were observed to be round with the microscope, the bottom of the bottle was gently tapped with hands and till the cells were fallen off from the wall of the bottle. 4 - 6 mL of fresh culture medium was added to the culture system for terminating the digestion. The cells were blown evenly, then divided into two new culture bottles, and another 12 - 14 mL of fresh culture medium was added to the bottles. After fully mixed, the bottles were placed in 37 ℃ and 5% CO2 incubator to incubate. When the cells were in logarithmic growth, the experiments can be performed.

3.2. Cell Group Processing and Collection

3.2.1. Cell Grouping

N2a cells at logarithmic phase were taken and inoculated in the T25 sterile cell culture bottle at a cell density of 1×105 per mL per bottle, 7mL per bottle, and incubated at 37 ℃ and 5% CO2 incubator for 12 h. After that, they were randomly divided into 7 groups: control group, PKA inhibitor H-89 group, model group, the Aβ25-35+H-89 group, the SBF low dose (1.125 mg/L) treatment group, the SBF medium dose (2.25 mg/L) treatment group and the SBF high dose (4.5 mg/L) treatment group. The control group contains 3% FBS and 97% high-glucose DMEM. The PKA inhibitor H-89 group is the

result of adding the H-89 of 48 nmol/L (final concentration) into the culture medium containing 3% FBS and 97% highglucose phenol red for 12 h. Model group makes Aβ25-35 have a final concentration of 40 mol/Lin 3% FBS and 97% high glucose phenol red culture medium and action 12 h. In the Aβ25-35+H-89 group,H-89 with a final concentration of 48 nmol/L was added into the culture medium which contains 3% FBS and 97% high glucose phenol red to act12 h, thenAβ25-35 with a final concentration of 40 μmol/L was added to act 12 h.SBF three dose treatment groups are to dissolve SBF in 3% FBS and 97% high glucose phenol red culture medium, so that the final concentration of SBF is respectively 1.125 mg/L, 2.25 mg/L and 4.5 mg/L, at the same time the H-89 with a final concentration of 48 nmol/L was added and action12 h, then the Aβ25-35 with a final concentration of 40 μmol/L was added to act for 12 h.

3.2.2. Cell Collection

Each bottle of cells was slowly blown with a 1 mL gun, and mixed, then transferred into a 10 mL centrifuge tube. At a speed of 5000 rpm/min, centrifugation for 10 min. The supernatant was discarded and 1.2 mL PBS was added. They were mixed evenly and then transferred into a 1.5 mL centrifuge tube. At a speed of 5000 rpm/min, centrifugation for 10 min. After the supernatant was discarded, the remaining part was sealed with a sealing film, and then stored in the -80 ℃ refrigerator for later use.

3.3. Extraction Protein and Protein Quantification

3.3.1. Extraction Protein

Each group of cells was taken out from the -80 ℃ refrigerator. They were placed on the ice box. 150 μL cracking liquid (RIPA: PMSF = 100:1) was added into each group of cells, the cells and the cracking liquid were thoroughly mixed with a gun. Mixture was cracking on the ice for 20 min, the sample after cracking was placed in 4 ℃ environment, centrifuged 20 min at a speed of 12000 rpm/min. After nucleic acids appear, ultrasound 4-6 times, the supernatant is protein.

3.3.2. BCA Method Protein Quantification

The standard protein and BCA work fluid was prepared according to the requirement, and the 96-well plates were taken out, the number of groups were set. First, 25 μL standard protein was added into each standard hole. In the second, 5 μL protein to be tested (set the double) was added to the control group and the other 6 groups in turn. Next, 20 μL PBS was added into each group in turn. Finally, 200 μL BCA working liquid was added into all the holes, and the 96-well plates werecovered with the lid, placed in 37 ℃ thermostat incubation for 30 min. After the incubation time was up,96-well plates weretaken out, and wait until it was cooled to room temperature, the absorbance value at 562 nm was measured with a microplate reader. A standard curve was drawn with Excel to calculate the protein concentration in the sample. The remaining samples was mixed with 5× protein loading sample buffer at a ratio of 4:1, modified with 100 ℃ boiling water for 5 min. After the mixture was cooled to room temperature, stored in the -20 ℃ refrigerator for later use.

3.4. Western Blot Method Detects the Protein Expression Level

Western blot was used to detect Tau protein expression levels at Ser199, Ser214, Ser404 and Thr231 sites and PKA protein expression levels in each group of N2a cells. Separation by polyacrylamide gel electrophoresis, and then transfer membrane. The PVDF membrane was closed in TBST of 5% skimmed milk powder for 2h, the primary antibody was diluted with 2 mL TBST, Ser199 (1:15) 000, Ser214 (1:500), Ser404 (1:500) and Thr231 (1:500) were placed in the 4 ℃ refrigerator overnight, incubated for 5 h at room temperature the next day. Goat anti-rabbit secondary antibody was diluted with 2 mL TBST (1:5000) and incubated at room temperature for an hour and a half. The membrane was placed in the 2 mL developer medium that was prepared in dark light to react for 3 min. And then start the exposure, the time is 3 s, 10 s, 30 s, 60 s, 120 s, respectively. After development, the strip was saved and analyzed with the IBM SPSS Statistics 21 statistical software, and the ratio of gray value of the target protein to β-actin protein was used as the relative expression amount of the target protein.

3.5. Statistical Analysis

The results of the study were statistically analyzed by IBM SPSS Statistics 21, expressed as means ±standard deviation (Means±SD). Multiple groups of samples means were compared by one-way ANOVA, the pairwise comparison between means was conducted by the method of least significant difference (LSD). The P<0.05 difference was statistically significant.

4. RESULTS

4.1. Effect of SBF on the Expression of PKA Abnormal Protein Induced by $A\beta_{25-35}$ in N2a Cells

As shown in Figure 1, compared with the control group, the PKA protein expression level in N2a cells in the H-89 group was decreased by 23.77% (p>0.05), and the model group protein expression level increased by 1.88 times (p<0.01). Compared with the model group, the protein expression level of $A\beta_{25-35}$+H-89 was decreased by11.64% (p<0.01). Compared with $A\beta_{25-35}$+H-89 group, the low dose group expression level of SBF decreased by 20.91% (p>0.05), and the expression level of SBF medium and high dose groups increased by8.41% (p>0.05) and 5.72% (p>0.05), respectively.

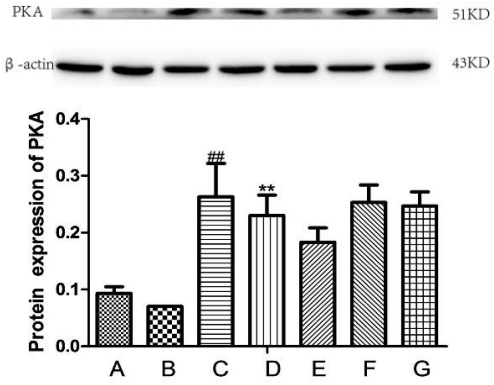

Figure1. *The effect of SBF on the protein expression of PKA in the N2a cell by composited $A\beta_{25-35}$. A: control group; B: PKA inhibitor H-89 group; C: model group; D: $A\beta_{25-35}$+ H-89group; E: SBF (1.125 mg/L) +$A\beta_{25-35}$ + H-89 group; F: SBF (2.25 mg/L) + $A\beta_{25-35}$ + H-89 group; G: SBF (4.5 mg/L) + $A\beta_{25-35}$ + H-89 group. Mean ± SD. n=2. $^{\#\#}p<0.01$ vs control group; $^{**}p<0.01$ vs $A\beta_{25-35}$ + H-89 group.*

4.2. Effect of SBF on the Expression of Phosphorylated Tau Protein at Ser199 Site Induced by Aβ25-35in N2a Cells

As shown in Figure 2, compared with the control group, the expression level of phosphorylated Tau protein at Ser199 site in N2a cells increased by 95.61% (p<0.01) in the H-89 group, and the model group protein expression level increased by 1.18 times (p<0.01). Compared with the model group, the expression level of Aβ25-35+H-89 group was decreased by 22.31% (p<0.01). Compared with Aβ25-35+H-89 group, the expression levels of SBF low and medium dose groups respectivelydecreased by 22.34% (p<0.01) and 29.63% (p<0.01), while the SBF high dose group expression level increased by 4.55% (p>0.05).

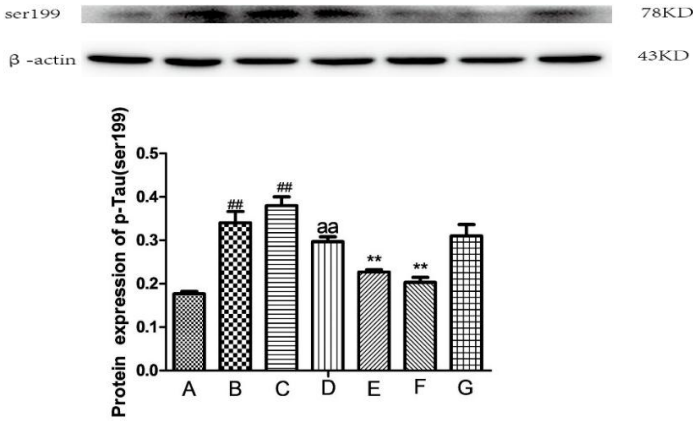

Figure2. *The effect of SBF on the protein expression of p-Tau (Ser199) in the N2a cell by composited. A: control group; B: PKA inhibitor H-89 group; C: model group; D: $A\beta_{25-35}$+ H-89 group; E: SBF (1.125 mg/L) + $A\beta_{25-35}$ + H-89 group; F: SBF (2.25 mg/L) + $A\beta_{25-35}$ + H-89 group; G: SBF (4.5 mg/L) + $A\beta_{25-35}$ + H-89 group. Mean ± SD. n =3. $^{\#\#}p<0.01$ vs control group; $^{aa}p<0.01$ vs model group; $^{**}p<0.01$ vs $A\beta_{25-35}$ + H-89 group.*

4.3. Effect of SBF on the Expression of Phosphorylated Tau Protein at Ser214 Site Induced by Aβ25-35 in N2a Cells

As shown in Figure 3, compared with the control group, the expression level of phosphorylated Tau protein at Ser214 site in N2a cells increased by 32.32% ($p<0.01$) in the H-89 group, and the model group protein expression level increased by 53.81% ($p<0.01$). Compared with the model group, the expression level of Aβ25-35+H-89 group was decreased by 18.82% ($p>0.05$). Compared with Aβ25-35+H-89, the expression levels of SBF three dose groups were respectivelydecreased by 28.29%, 20.02% and 34.22%, with significant significance ($p<0.01$).

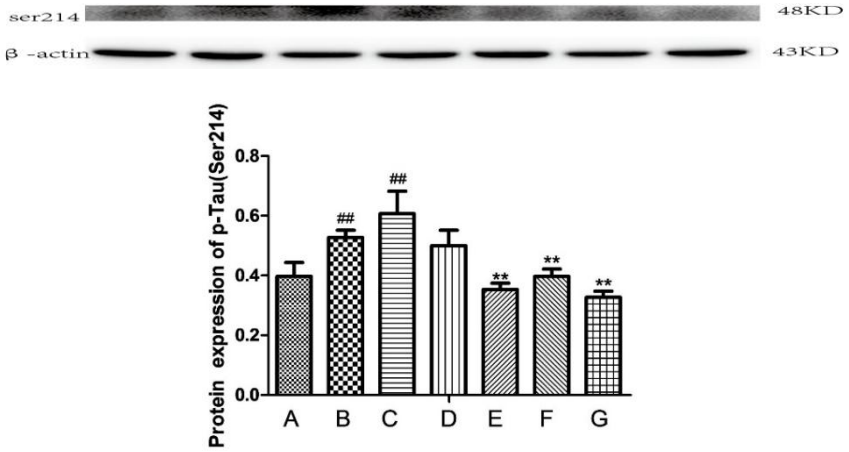

Figure3. *The effect of SBF on the protein expression of p-Tau(Ser214) in the N2a cell by composited. A: control group; B: PKA inhibitor H-89 group; C: model group; D: $A\beta_{25-35}$+ H-89 group; E: SBF (1.125 mg/L) + $A\beta_{25-35}$ + H-89 group; F: SBF (2.25 mg/L) + $A\beta_{25-35}$ + H-89 group; G: SBF (4.5 mg/L) + $A\beta_{25-35}$ + H-89 group. Mean ± SD. n =2. $^{\#\#}p<0.01$ vs control group;$^{**}p<0.01$ vs $A\beta_{25-35}$ + H-89 group.*

4.4. Effect of SBF on the Expression of Phosphorylated Tau Protein at Ser404 Site Induced by Aβ25-35 in N2a Cells

As shown in Figure 4, compared with the control group, the expression level of phosphorylated Tau protein at Ser404 site in N2a cells in the H-89 group increased by 20.94% ($p<0.01$), and the model group increased by 31.66% ($p<0.01$). Compared with the model group, the expression level of Aβ25-35+H-89 group was decreased by 26.87% ($p<0.01$). Compared with Aβ25-35+H-89 group, the SBF low dose group expression level increased by 2.15% ($p>0.05$), while the expression levels of SBF low and medium dose groups decreased by 2.49% and 13.57% ($p>0.05$), respectively.

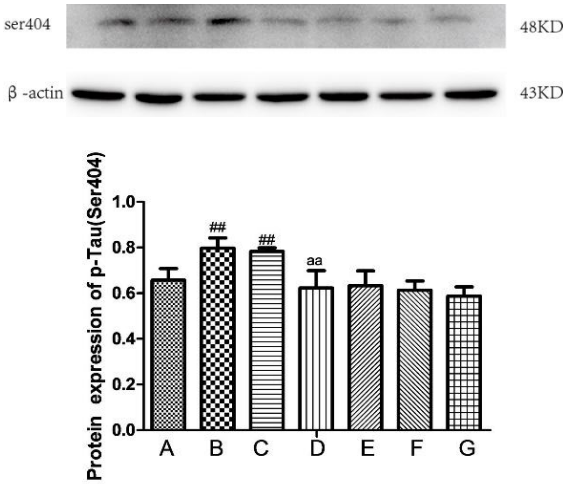

Figure4. *The effect of SBF on the protein expression of p-Tau(Ser404) in the N2a cell by composited. A: control group; B: PKA inhibitor H-89 group; C: model group; D: $A\beta_{25-35}$+ H-89 group; E: SBF (1.125 mg/L) + $A\beta_{25-35}$ + H-89 group; F: SBF (2.25 mg/L) + $A\beta_{25-35}$ + H-89 group; G: SBF (4.5 mg/L) + $A\beta_{25-35}$ + H-89 group. Mean ± SD. n =2. $^{\#\#}p<0.01$ vs control group;aap <0.01 vs model group.*

4.5. Effect of SBF on Expression of Phosphorylated Tau Protein at Thr231 Site by Aβ25-35 in N2a Cells

As shown in Figure 5, compared with the control group, the expression level of phosphorylated Tau protein at Thr231 site in N2a cells increased by 32.24% ($p<0.01$) of the H-89 group, and the model group protein expression level increased by 54.19% ($p<0.01$). Compared with the model group, the expression level of Aβ25-35+H-89 group was decreased by 21.92% ($p<0.05$). Compared with Aβ25-35+H-89 group, SBF low dose group expression level decreased by 7.10% ($p>0.05$), and the expression levels of SBF medium and high dose groups increased by 16.86% ($p<0.01$) and 5.79% ($p>0.05$), respectively.

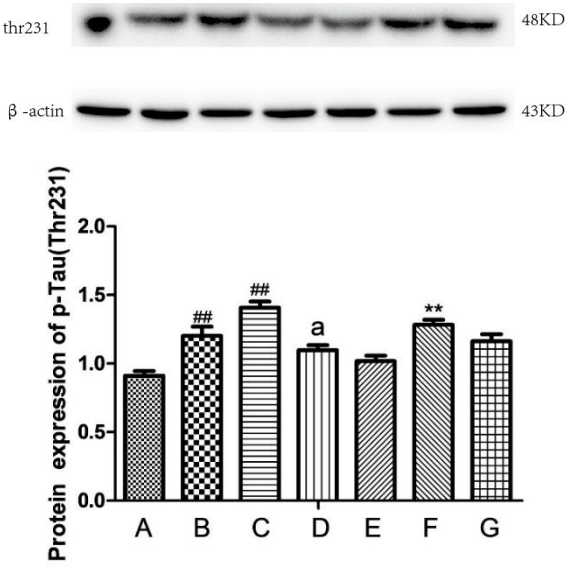

Figure5. *The effect of SBF on the protein expression of p-Tau(Thr231) in the N2a cell by composited. A: control group; B: PKA inhibitor H-89 group; C: model group; D: $A\beta_{25-35}$+ H-89 group; E: SBF (1.125 mg/L) + $A\beta_{25-35}$ + H-89 group; F: SBF (2.25 mg/L) + $A\beta_{25-35}$ + H-89 group; G: SBF (4.5 mg/L) + $A\beta_{25-35}$ + H-89 group. Mean ± SD. n =2. $^{\#\#}p<0.01$ vs control group;$^{a}p<0.05$ vs model group; $^{**}p<0.01$ vs $A\beta_{25-35}$ + H-89 group*

5. DISCUSSION

Tau protein is a kind of microtubule associated protein, can combine early with tubulin to form early microtubule core, normal Tau protein has 2-3 phosphate groups. However, the Tau protein in the brain of AD patients will appear hyperphosphorylated phenomenon, the content of which is 3-4 times higher than the normal content. And the combine of phosphorylated Tau protein with microtubule-related protein will be affected, causing hyperphosphorylated Tau protein to fall off and aggregate to form the NFT which leads to produce neurotoxicity [7-8]. Aβ is a protein that produced by the hydrolysis of β-Amyloid precursor protein (APP), which is widely existing in various tissues of the body. It has strong self-aggregation and could easily form refractory precipitate [9]. The Aβ cascade hypothesis suggests that Aβ has neurotoxicity and can promote the phosphorylated Tau protein, especially the aggregative Aβ which can make the phosphorylated Tau protein more serious [10]. Tau protein depends on the catalytic action of multiple protein kinases. There are multiple protein kinases in nerve cells, including cAMP-dependent kinase (PKA), glycogen synthesekinase-3β (GSK3β), cyclin-dependent kinase 5 (CDK5) and Ca-CaM dependent protein kinase II (Ca2+/ CaMK II), etc. These kinases all can phosphorylate Tau protein in vitro [11]. PKA is a protein kinaseguided by non-proline, can effectively catalyze Tau protein phosphorylation at Ser214, Ser262, Ser409 and Ser356 sites in vitro [12]. PKA mediated Tau protein phosphorylation can trigger that GSK3β mediates a series of phosphorylated Tau protein sites associated with AD, including p-Tau (Ser199), p-Tau (Ser202), p-Tau (Thr231) and p-Tau (Ser422) and so on, while also inhibiting other phosphorylated sites, such as Ser404 and Thr212. PKA itself can increase the activity of GSK3β and CDK5, PKA inhibits the phosphorylated sites Ser404 and Thr212, may be due to increased phosphorylated level at Tyr216 site of GSK3β. GSK3β or CDK5 can catalyze the phosphorylation of multiple sites of Tau protein, including Ser199, Ser202, Thr205, Thr212, Thr231, Ser396 and Ser404, etc. [13-14]. PKA signal transduction system is composed of receptors, GTP binding proteins and adenylate cyclase coupling.

In addition to directly causing Tau protein phosphorylation, PKA can also act together with other protein kinases to regulate the phosphorylated Tau protein. The activities of GSK3β and CDK5 pretreated by PKA were significantly enhanced [15]. Many factors have effects on the activity of PKA, among which H-89, as a highly selective PKA catalytic subunit inhibitor, can bind to the catalytic subunit of PKAto inhibit the activity of PKA [16], so it can be used as a tool drug to investigate the effect of PKA on Tau protein phosphorylation.

The results of thepresent study showed that the protein expression level of PKA in N2a cells was significantly decreasedafter the application of H-89, indicating that PKA activity was decreased, further indicated that H-89 can inhibit the activity of PKA protein. The protein expression level of PKA in N2a cells of Aβ group was significantly increased, indicating that Aβ can enhance the PKA activity, so that Aβ promotes Tau protein hyperphosphorylation induced by PKA. These are consistent with the results that increased cell mortality and reduced survival rate in group Aβ detected in the previous experiment. The expression level of PKA protein in N2a cells of group $A\beta_{25-35}$+H-89 was decreased, indicating that H-89 has an inhibitory effect on the increase of PKA activity in N2a cells induced by $A\beta_{25-35}$, thus reducing the phosphorylated level of Tau protein. SBF three dose treatment groups have appeared the situation that the expression level of PKA protein in N2a cells decreased at low dose group and increased at medium and high dose group, indicating that lowdose SBF could reverse the increase of PKA and Tau protein phosphorylation expression levelinduced by $A\beta_{25-35}$ in N2a cells, while the medium and highdose SBF failed to reverse the increaseof PKAprotein expression level induced by $A\beta_{25-35}$.The results are related to the chemical composition of the mixture of other multiple ingredients contained in SBF, also consistent with the traditional Chinese medicine (TCM) activity which often appears inverted U-shape.

The studies showed that the phosphorylated Tau protein was increased at Ser199, Ser214, Ser404 and Thr231 sites after the application of H-89, indicating that H-89 failed to inhibit the activity of PKA and reduced Tau protein phosphorylation at these sites. The reason may be that Tau protein was made to phosphorylate by GSK3β or CDK5 at these sites. The expression levels of Tau protein at Ser199, Ser214, Ser404 and Thr231 sites in N2a cells were also increased of Aβgroup, indicating that PKA activity is enhanced and promoted the hyperphosphorylatedTau protein, especially at Ser404 site, Ser409 site of PKA hyprephosphorylation can enhance the phosphorylation of GSK3β for Ser404 site, resulting in a significant increase in phosphorylation expression level. After application of H-89 in group Aβ, Tau protein expression levels at Ser199, Ser214, Ser404 and Thr231 sites all decreased, indicating that PKA activity was decreased and H-89 can reduce the activity of PKA, and then reduce the hyperphosphorylation expression level ofTau protein.

The expression levels of Tau protein in the SBF three dose treatment groups appeared inverted U-shape at Ser199, Ser214 and Thr231 sites, indicating that lowdose SBF has a reduced effect on Tau protein hyperphosphorylation at these three sites,while highdose SBF failed to reverse theincrease of theTau proteinphosphorylation expression level caused by $A\beta_{25-35}$in N2a cells, which may be related to the increased phosphorylation expression level of Tau protein or the increased activity of CDK5 at the Ser199 and Tyr216 phosphorylationsites of GSK3β. The anti-Tau protein antibody that only recognizes the phosphorylation site of PKA was used to find that the phosphorylation of Ser214 site has existed in the early stage which AD neurons without tangles, and participated in the whole entanglement process.It suggests that PKA has played a role in the phosphorylation of the Ser214 site of Tau protein in the early stage that AD neuron degeneration. In addition, Tau phosphorylation of Ser214 site can promote the phosphorylation of Thr231 site, the reason is that both overexpressed GSK3β or CDK5 can increase the phosphorylated Tau protein at Ser214. Both enzymes up-regulate PKA activity and indirectly excessive increase the phosphorylation of Ser214 at the same time, thereby promoting phosphorylation of Thr231 site. It was found that SBF significantly decreased the phosphorylation level of Tau protein and presented a dose-dependent relationship at the site of Ser404.

Combined with previous studies, this present study suggests that SBF can reverse the increase of the Tau proteinphosphorylation level which caused by the $A\beta_{25-35}$in N2a cells.It is achieved by regulating the activity of PKA. It also confirms that SBF can prevent the abnormal elevation of phosphorylation level in brain of AD patients, which provides a new target drug for clinical treatment of AD. Whether SBF has other mechanisms of action on AD,still needs further studies and discussion.

ACKNOWLEDGEMENTS

We acknowledge financial support by Hebei Provincial Natural Science Foundation (No. C2009001007, H2014406048) and Hebei Provincial Administration of Traditional Chinese Medicine (No. 05027, 2014062) and Key Subject Construction Project of Hebei Provincial College of China.

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Citation: *Yazhen, S. et al.,(2019). Effect of SBF on Aβ25-35 Induced- Abnormal Changes of PKA and tau Protein Phosphorylationin N2a Cells. International Journal of Medicinal Plants and Natural Products (IJMPNP), 5(1), pp.8-15.http://dx.doi.org/10.20431/2454-7999.0501002*