이학석사학위논문

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2014 년 7 월

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Protective effect of Tat protein transduction domain-heat shock protein 27 fusion protein on neurodegeneration

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A Thesis for the M.S. Degree in Biochemistry

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Abstract

Alzheimer's disease (AD) is an age-related disorder that causes a loss of brain function. Hyperphosphorylation of tau and the subsequent formation of intracellular neurofibrillary tangles (NFTs) are implicated in the pathogenesis of AD. Hyperphosphorylated tau accumulates into insoluble paired helical filaments that aggregate into NFTs; therefore, regulation of tau phosphorylation represents an important treatment approach for AD. Heat shock protein 27 (Hsp27) plays a specific role in human neurodegenerative diseases; however, few studies have examined its therapeutic effect. In this study, we induced tau hyperphosphorylation using okadaic acid, which is a protein phosphatase inhibitor, and generated a fusion protein of Hsp27 and the protein transduction domain of the HIV Tat protein (Tat-Hsp27) to enhance the delivery of Hsp27. Tat-Hsp27 was delivered into SH-SY5Y neuroblastoma cells following a 2 h treatment, and the transduction level was proportional to the Tat-Hsp27 concentration. Additionally, Tat-Hsp27 reduced the level of hyperphosphorylated tau and protected cells from apoptotic cell death caused by abnormal tau adjajggregates. These results reveal that Hsp27 represents a valuable protein therapeutic for AD.

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1. Introduction

Alzheimer's disease (AD) is a progressive neurological disorder that causes memory loss. Various pathological hallmarks of AD include synaptic and neuronal loss, amyloid plaques primarily composed of the 42-residue hydrophobic β-amyloid peptide (Aβ) [1] and neurofibrillary tangles (NFTs) composed of aggregates of hyperphosphorylated tau, which is a microtubule-associated protein [2]. Amyloid plaques and NFTs are considered the primary factors involved in the pathogenesis of AD [3]. Although previous studies have primarily focused on the role of β-amyloid peptides, recent studies on the role of tau in AD pathogenesis have indicated that hyperphosphorylated tau aggregates into insoluble paired helical filaments (PHFs), which induce neuronal dysfunction [4]. Moreover, these two pathological hallmarks demonstrate synergistic effects on synaptic dysfunction [5, 6].

As a microtubule-associated protein, aggregates of normal tau protect cells against toxic hyperphosphorylation, although neuronal death occurred following a period of survival [7]. However, tau is not only abnormally phosphorylated but also aggregates into insoluble forms such as PHFs and NFTs [8, 9] in various AD mouse models [10-12]. Therefore, an approach that reduces the level of hyperphosphorylated

tau would represent a valuable treatment for AD [13]. Tau can bind heat shock proteins (HSPs), which trigger the recruitment of CHIP (which is a co-chaperone that exhibits E3 activity), to the complex. CHIP induces the ubiquitination of tau and activates its degradation when tau is defective [14]. Hsp27 directly associates with hyperphosphorylated tau or PHFs and regulates cell survival by eliminating tau aggregates [15].

Heat shock proteins are induced in response to cellular stress as molecular chaperones that inhibit protein aggregation [16]. HSPs can prevent apoptosis and increase cell viability during cellular stress [17, 18]. HSPs are also critical regulators of normal neural physiological function and cell stress responses [19-21]. However, the therapeutic effects of exogenous Hsp27 on disease models have not been investigated. Therefore we sought to determine whether Hsp27 can reduce hyperphosphorylated tau in AD-induced SH-SY5Y cells.

To more effectively deliver Hsp27 into cells, we combined the HIV protein transduction domain (PTD) Tat with the Hsp27 protein (Tat-Hsp27). HIV Tat (11 residues, YGRKKRRQRRR), can rapidly transduce into cells [22] and deliver full-length proteins into cells [23]. As expected, Tat-Hsp27 effectively reduced the phosphorylation of tau and rescued the cell death caused by abnormal tau aggregates. Therefore, our study suggests that Tat-Hsp27 may represent a potential protein therapeutic for tau-induced neurodegeneration.

2. Materials and Methods

Construction of the expression vector

We designed a bacterial expression vector (His6-Tat-Hsp27) containing hexahistidine leader sequence, 11-amino acid Tat PTD sequence (YGRKKRRQRRR), and Hsp27 protein sequence. The Hsp27 fragment (BD Bioscience) was generated by PCR using human cDNA as a template and oligonucleotide primers containing BamHI and XhoI restriction sites. The pET-28a vector (Novagen) and the Tat PTD were digested with NdeI and BamHI and ligated. The ligated vector was transformed into chemically competent DH5α E. coli cells (Enzynomics, Korea). After purification of the plasmid containing the Tat PTD, the plasmid and the Hsp27 fragment were digested with BamHI and XhoI and ligated. The recombinant plasmid (pET21a-Tat-Hsp27) was transformed into competent DH5α E. coli cells for plasmid purification.

Expression and purification of Hsp27

The recombinant plasmid (His6-Tat-Hsp27) was transformed into the E. coli strain BL21(DE3) for protein expression. Transformed cells were plated on an LB agar (Merck) plate containing kanamycin (Sigma-Aldrich) and incubated overnight at 37℃. Two hundred

milliliters of LB medium containing 1 mM kanamycin was inoculated with a single colony and incubated overnight at 37℃ with shaking at 200 rpm. The following day, 1 L of LB medium was inoculated with this preculture and incubated at 37℃ until an OD600 of 0.5. Protein expression was induced by the addition of Isopropyl β-D-1-thiogalactopyranoside (MB cell, Korea) at a final concentration of 1 mM, and the cells were incubated at 37℃ for an additional 4 h. The cells were centrifuged at 6,000 rpm for 15 min at 4℃ and resuspended in binding buffer (20 mM Tris-HCl, 500 mM NaCl, 35 mM imidazole, pH 7.5). Cells were lysed by sonication on ice using a sonicator (Sonics Vibra-Cell VCX 750, Sonic & Materials Inc., USA) with 1-s pulses and 8-s pauses for 30 min. After sonication, the lysates were centrifuged at 10,000 rpm for 15 min. The clarified lysate was loaded onto a pre-equilibrated HisTrap HP column (GE Healthcare). His-tagged Tat-Hsp27 protein was eluted with elution buffer (20 mM Tris-HCl, 500 mM NaCl, 1 M imidazole, pH 7.5). His-tagged Tat-Hsp27 was further purified using size exclusion chromatography on a HiLoad 16/600 Superdex 200 prep grade column (GE Healthcare) using 20 mM Tris-HCl (pH 7.5) and 100 mM NaCl. For the detection of Tat-Hsp27 delivery into cells, we conjugated FITC to Tat-Hsp27 using an FITC labeling kit (Thermo Scientific). The concentration of protein was determined using BCA protein assay kit (Pierce).

Cell culture

SH-SY5Y human neuroblastoma cells were grown in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic under an atmosphere of 5% CO_2 and 95% air (all from WELGENE). The medium was refreshed every three days. Cells below passage 24 were used for experiments.

Western blot analysis

For Western blot analysis, the cells treated with Tat-Hsp27 and okadaic acid were washed in DPBS and harvested in RIPA buffer (Pierce). To prevent from further phosphorylation, we added Protease inhibitor cocktail and phosphatase inhibitor cocktail (both from Sigma-Aldrich) into the cell lysates. Lysates were then centrifuged at 15,000 x g for 15 min at 4℃. The supernatant was separated in a 12% SDS-PAGE gel, and the proteins were transferred to a nitrocellulose membrane. After blocking in 5% skim milk/TBST (Tris-buffered saline and 0.1% Tween 20, pH 7.5), the membrane was washed in TBST three times (15 min each) and incubated with primary antibody overnight at 4℃. The membrane was subsequently washed and incubated with the appropriate secondary antibody (HRP conjugated goat-anti mouse and

goat-anti rabbit) for 2 h at room temperature. Peroxidase-linked anti-rabbit and anti-mouse IgG were purchased from Santa Cruz Biotechnology (USA). Rabbit polyclonal anti-tau phosphoserine 199/202 antibody and mouse monoclonal anti-tau-1 antibody were obtained from Millipore (USA). Anti-human tau monoclonal antibody and rabbit anti-Hsp27 polyclonal antibody were purchased from Pierce Biotechnology (USA).

Immunocytochemistry

The SH-SY5Y cells were seeded on 2-well slides (Lab-Tek chamber, Nalge Nunc, NY) at a density of 5.0×10^5 per well, treated with 2 μM Tat-Hsp27 for 2 h, and then treated with 10 nM okadaic acid for 14 h. The cells were rinsed two times with PBS and fixed with 4% paraformaldehyde for 30 min. The cells were subsequently permeabilized for 10 min with 0.5% Triton X-100 followed by three 5-min washes in PBS. To reduce nonspecific binding, we used PBS containing 0.5% bovine serum albumin as blocking buffer. After incubation with the blocking buffer for 1 h, the cells were incubated with the primary antibody AT8 (anti-phosphorylated-tau antibody) for 4 h, followed by three 5-min washes. The cells were subsequently incubated with goat anti-rabbit (H+L) FITC-conjugated antibody. After

washing with PBS, cells were imaged using an image restoration microscope (Applied Precision, USA).

Cell viability/cytotoxicity assay

A cytotoxicity assay was performed using a Cell Counting Kit-8 (Dojindo, Korea). SH-SY5Y cells were plated in a 96-well plate at 5.0×10^4 in 100 μL of RPMI 1640 medium containing 10% FBS. After incubation for 48 h, cells were treated with 0.5 μM or 2 μM Tat-Hsp27 for 2 h. Subsequently, various concentrations of okadaic acid were added for 8 h to induce abnormally phosphorylated tau. Ten microliters of CCK-8 solution was added, and the cells were incubated at 37℃ for 2 h. The absorbance at 450 nm was measured using a microplate reader (Molecular Devices Co., Menlo Park, CA).

In situ terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

SH-SY5Y cells were seeded on a 2-well slide at a density of 5.0×10^5 per well, treated with 2 μM Tat-Hsp27 for 2 h, and then treated with 10 nM okadaic acid for 14 h. The cells were fixed by immersion in PBS containing 4% formaldehyde (pH 7.4). After washing with PBS, the

cells were permeabilized in 0.2% Triton X-100 and washed again. After treatment with equilibration buffer, the cells were added to rTdT incubation buffer containing nucleotide mix. To terminate the reaction, saline-sodium citrate buffer was added to the cells; nuclei were stained with VECTASHIELD + DAPI. The cells were analyzed under a fluorescence microscope at 520 nm (green fluorescence) and 460 nm (blue, DAPI).

Statistical analysis

The data are expressed as the mean ± SE and were calculated using an unpaired Student's t-test. A value of $p < 0.05$ indicates a significant difference.

3. Results

The sequence encoding Tat-Hsp27 fusion protein was cloned into the pET28a vector, which produces a recombinant protein with a hexahistidine tag (Fig.1A) and purified using affinity purification and size exclusion chromatography. Fractions containing His-tagged Tat-Hsp27 were identified using 12% SDS-PAGE analysis with Coomassie Brilliant Blue staining. His-tagged Tat-Hsp27 in fractions 1-6 exhibited

high affinity for the affinity matrix. The eluted His-tagged Tat-Hsp27 was further purified using size exclusion chromatography. Fractions containing His-tagged Tat-Hsp27 were identified using 12% SDS-PAGE with Coomassie Brilliant Blue staining. His-tagged Tat-Hsp27 eluted as a single peak from size exclusion chromatography, as confirmed by Western blot analysis (Fig. 1D), and migrated to a position slightly above the 25-kDa molecular weight marker in 12% SDS-PAGE analysis. The purified recombinant His-tagged Tat-Hsp27 was concentrated to 1.438 mg/mL as determined using the BCA assay.

To investigate whether Tat-Hsp27 could be effectively delivered into cells, we conjugated FITC to Tat-Hsp27. Image restoration microscopy indicated that FITC-Tat-Hsp27 was efficiently delivered into SH-SY5Y cells following treatment for 2 h at a concentration of 2 μM, in contrast to 2 μM wt-Hsp27. We also performed Western blot analysis to confirm the intracellular delivery of Tat-Hsp27 and to determine whether its transduction is dependent on the Tat-Hsp27 concentration. SH-SY5Y cells were treated with Tat-Hsp27 for 2 h and were lysed using RIPA buffer. As shown in Figure 2B, Hsp27 was present in the lysate of SH-SY5Y cells treated with 2 and 5 μM Tat-Hsp27, whereas equivalent concentrations of wt-Hsp27 did not transduce cells. Additionally, increasing the concentration of Tat-Hsp27 used to treat cells resulted in an increased level of delivered protein. Therefore, the

recombinant Tat-Hsp27 can be delivered into cells in a concentration-dependent manner.

Because Hsp27 is known to have an effect on phosphorylation, we investigated whether Hsp27 can reduce the level of hyperphosphorylated tau, which is implicated in the pathogenesis of AD. SH-SY5Y cells were treated with 2 or 5 μM Tat-Hsp27 for 2 h, and the phosphorylation of normal tau was induced by the addition of 50 or 100 nM okadaic acid. To quantify the relative phosphorylation, we normalized the level of phosphorylated tau (p-tau) in Western blot analysis to the level of β-actin using SigmaPlot software. Following treatment with 50 or 100 nM okadaic acid for 2 h, the level of phosphorylated tau was approximately 1.5-fold or 2-fold greater than the control group, respectively. Following treatment with 50 nM okadaic acid, the level of p-tau in cells pretreated with 2 μM and 5 μM Tat-Hsp27 decreased with an increasing concentration of Tat-Hsp27 (Fig. 3). In contrast, following treatment with 100 nM okadaic acid, cells pretreated with 5 μM Tat-Hsp27 contained slightly higher levels of p-tau than cells pretreated with 2 μM Tat-Hsp27. This finding suggest that when tau is hyperphosphorylated, a high concentration of Tat-Hsp27 has a negative effect on the reduction in hyperphosphorylated tau. Therefore, we used 2 μM Tat-Hsp27 in subsequent experiments. We also performed immunocytochemistry

using an anti-p-tau antibody conjugated to FITC (Fig. 4). SH-SY5Y cells were treated with 2 μM Tat-Hsp27 for 2 h and were subsequently treated with 10 nM okadaic acid for 14 h. The level of p-tau was significantly decreased compared with treatment with okadaic acid alone (n = 3).

A cytotoxicity assay was performed to determine whether Tat-Hsp27 directly affected the cell death induced by hyperphosphorylated tau. We first evaluated the relative cell viability (RCV) in the presence of Tat-Hsp27 alone. As shown in Figure 5A, treatment with Tat-Hsp27 alone did not alter the RCV of normal cells. In contrast, SH-SY5Y cells treated with 500 nM okadaic acid for 4 h exhibited a decrease in the RCV of approximately 65% (Fig. 5B). When cells were treated with 0.5 μM or 2 μM Tat-Hsp27 followed by treatment with 500 nM okadaic acid, the RCV increased to approximately 96% and 92%, respectively. Although treatment with a higher concentration of Tat-Hsp27 appeared to demonstrate a slightly negative effect on the cell death induced by hyperphosphorylated tau, Tat-Hsp27 clearly demonstrated a protective effect against this cell death.

Treatment with 10 nM okadaic acid alone for 14 h greatly increased the number of TUNEL-positive cells, whereas the cells pretreated with 2 μM Tat-Hsp27 exhibited decreased TUNEL positivity (Fig. 6A, 6B).

In the control group, apoptotic cells comprised 2.29% of the total number of cells. Following exposure to 10 nM okadaic acid, the percentage of apoptotic cells increased to 17.84%. However, this increase was inhibited by Tat-Hsp27 (4.56%). Therefore, intracellular delivery of Tat-Hsp27 prevented the apoptotic cell death induced by hyperphosphorylated tau.

4. Discussion

In this study, the recombinant Tat-Hsp27 reduced the level of hyperphosphorylated tau induced by okadaic acid in SH-SY5Y neuroblastoma cells and prevented the apoptosis induced by abnormal tau aggregates in our cellular model of AD. The effect of Hsp27 on phosphorylated tau has recently received increasing attention. However, few studies have examined the therapeutic effect of Hsp27 on hyperphosphorylated tau, which has been implicated in the pathogenesis of AD. In this study, we demonstrate that Hsp27 exhibits a protective effect on apoptotic cell death caused by pathological tau. This finding suggests that Hsp27 may represent a potential protein therapeutic for AD.

Tau stabilizes microtubules; however, tau mutations that result in its hyperphosphorylation lead to the formation of tau filaments that can form twisted ribbons or rope-like filaments [2]. Tau is primarily phosphorylated and dephosphorylates at least thirty serine or threonine sites [13]. Because the phosphorylation of tau is regulated by various kinases, such as proline-directed protein kinases and glycogen synthase kinase 3, and phosphatases, such as Ser/Thr protein phosphatases 1, 2A, 2B (calcineurin) and 2C, we used okadaic acid, which is a protein phosphatase inhibitor, to trigger PHF-like hyperphosphorylation of tau [24]. We used the AT8 antibody to detect phosphorylated tau at Ser214 and Thr205 [25]. Okadaic acid inhibits Ser/Thr protein phosphatases and can induce tau hyperphosphorylation and neurodegeneration [26]. In AD, second to β-amyloid, tauopathy has been found to correlate with cognitive decline [27]. Tauopathy is prominent in all cases of early-onset familial AD that are by definition amyloidogenic in origin because of mutations in the amyloid precursor protein [28]. A recent study reported that hyperphosphorylation of tau leads to a 20-fold inhibition of tau–tubulin binding affinity. This finding supports a critical role for tau in the pathogenesis of NFT-induced degeneration because the balance between kinases and phosphatases is disturbed in AD, resulting in the disassociation of tau from microtubules and its subsequent aggregation [29]. In our previous study, we demonstrated

that hyperphosphorylated tau, but not the overexpression of normal tau alone, reduces the cell viability of the neuroblastoma cell line SH-SY5Y [30]. Thus, in the present study, a cellular model of AD was induced by hyperphosphorylation of endogenous tau.

Moreover, we utilized the PTD of the HIV Tat protein to enhance the delivery of Hsp27, thereby enhancing its protective effect [19, 31]. Conjugation with specific peptide sequences, which are termed PTDs or cell-penetrating peptides, improves the delivery of a range of agents, including antisense oligonucleotides, plasmids, microbeads and liposomes, which suggests that these peptide sequences may represent a universal in vitro and in vivo cellular delivery system [23]. We confirmed that a fusion protein of Hsp27 and the Tat PTD was delivered into normal human neuroblastoma SH-SY5Y cells and into SH-SY5Y cells containing hyperphosphorylated tau induced by okadaic acid treatment. To determine whether Tat-Hsp27 demonstrates a protective effect against okadaic acid-induced cell death, SH-SY5Y cells containing hyperphosphorylated tau were treated with the fusion protein. The levels of phosphorylated tau significantly decreased compared with cells that were not treated with Tat-Hsp27, and the relative cell viability was enhanced. This result suggests that Tat-Hsp27 represents a potential protein therapeutic for the reduction of hyperphosphorylated tau.

Protein delivery systems offer several advantages, such as the ease of production from synthetic or natural compounds that provoke a low inflammatory response [32]. In comparison to other delivery systems, such as gene delivery that utilizes plasmid or viral vectors, our recombinant Tat-Hsp27 also offers several advantages. The straightforward purification of Tat-Hsp27 is amenable to large-scale production. Moreover, Tat-Hsp27 is nontoxic and stable and can be readily delivered into cells.

As previously mentioned, PTDs can readily and rapidly deliver proteins into cells. Drugs to treat neurodegenerative diseases must be capable of penetrating the blood-brain barrier (BBB) to be effectively delivered [33]. However, greater than 98% of all potential CNS drugs cannot cross the BBB [34]. Hence, we designed a recombinant Tat-Hsp27 fusion protein to treat a cellular AD model, and this fusion protein was delivered into cells at high levels within 2 h (Fig. 2). Therefore, we anticipate that uptake of Tat-Hsp27 into the brain will readily occur when nasally administered and will protect against the cell death caused by hyperphosphorylated tau, which is one of the primary causes of AD [34, 35]. Thus, we suggest that Tat-Hsp27 demonstrates a protective effect against hyperphosphorylated tau and may represent a valuable protein therapeutic for AD.

5. Conclusions

In this study, Tat protein transduction domain-heat shock protein 27 fusion protein (Tat-Hsp27) effectively reduced hyperphosphorylate tau, apoptotic cell death. Moreover, this Tat-Hsp27 is nontoxic and stable and also can be readily effectively delivered. Therefore, Tat-Hsp27 demonstrates a potential protein therapeutic for neurodegenerative disease.

6. References

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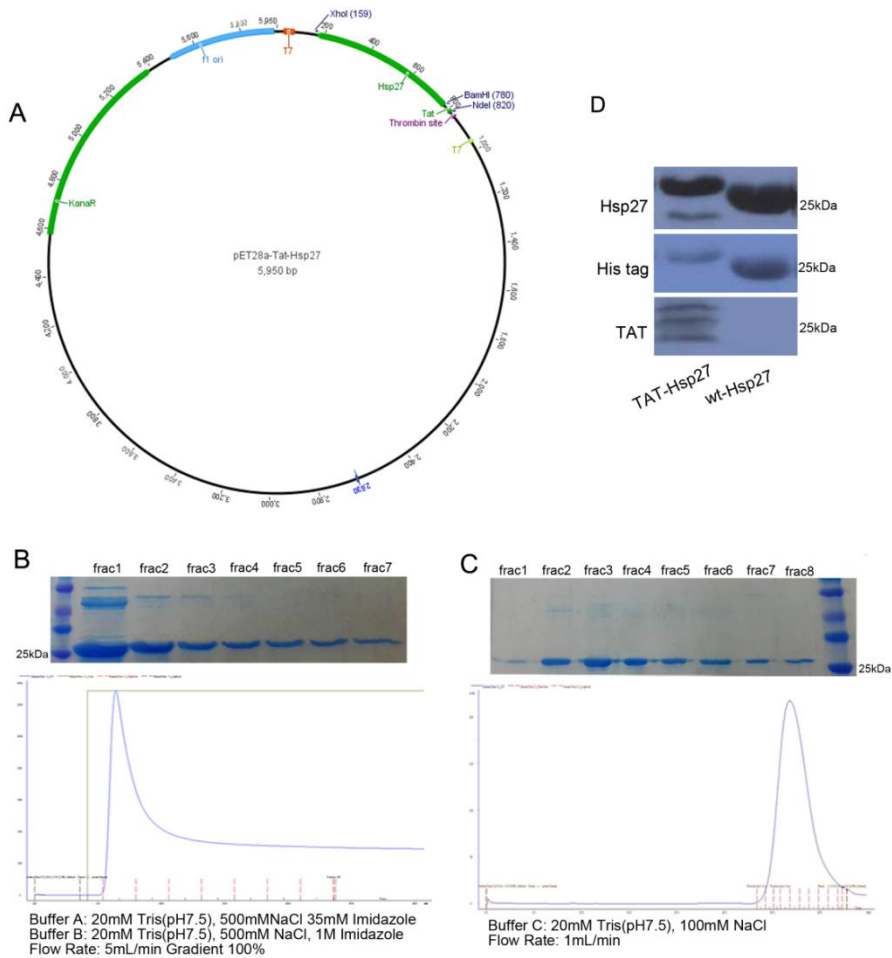
7. Figures

Figure 1. Purification of His-tagged Tat-Hsp27. (A) pET28a vector containing hexahistidine and Tat protein transduction domain and Hsp27 cDNA. (B) His-tagged Tat-Hsp27 was eluted from the affinity chromatography column using isocratic elusion buffer (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, and 1 M imidazole). (C) His-tagged Tat-Hsp27 was further purified using size exclusion chromatography (20

mM Tris-HCl, pH 7.5, and 100 mM NaCl) and eluted as a single peak. Fractions were analyzed using SDS-PAGE after each purification step. (D) Western blot analysis of purified His-tagged Tat-Hsp27 using anti-His tag, anti-Hsp27, and anti-Tat antibodies.
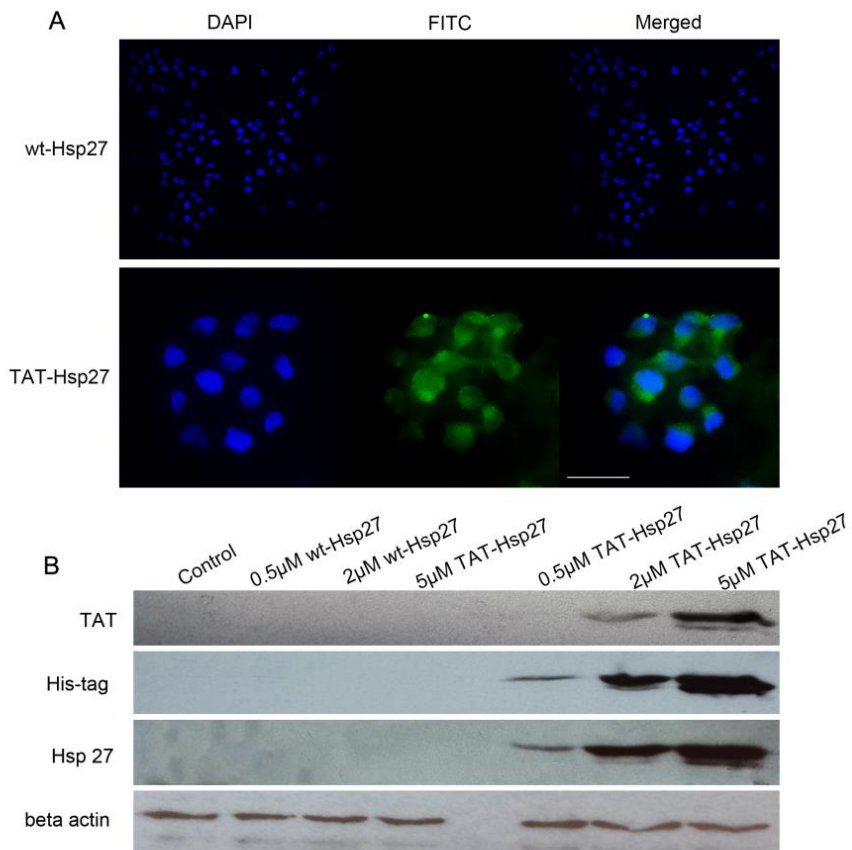
Figure 2. Transduction of wt-Hsp27 and Tat-Hsp27 into SH-SY5Y cells. (A) Immunocytochemistry. FITC-conjugated wt-Hsp27 or Tat-Hsp27 was delivered into cells and analyzed 2 h later. Image restoration microscopy indicates that Tat-Hsp27 was rapidly and efficiently delivered, in contrast to wt-Hsp27. Scale bar: 30 μm. (B) Protein from cell lysates of SH-SY5Y cells treated with wt-Hsp27 or Tat-Hsp27 was analyzed using Western blot analysis. The protein expression was normalized to actin.
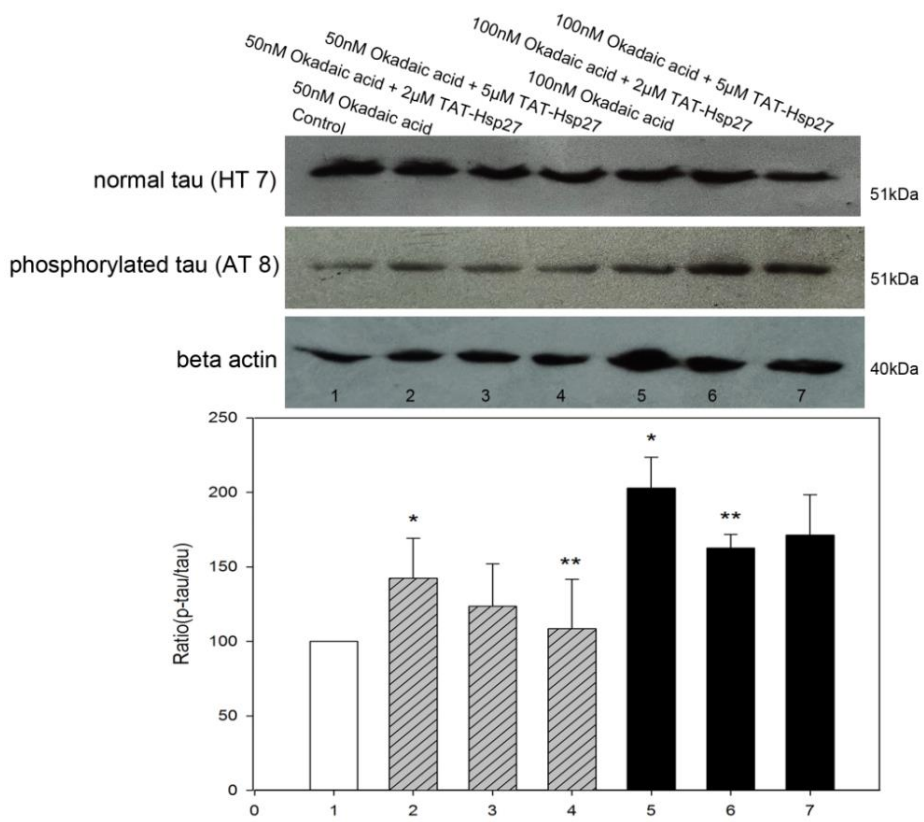
Figure 3. Effect of Tat-Hsp27 on tau hyperphosphorylation. Western blot analysis of normal tau and phosphorylated tau, with β-actin as a loading control. *p < 0.05 compared with the control. **p < 0.05 compared with okadaic acid alone. The data are presented as the mean ± SD (n = 3).
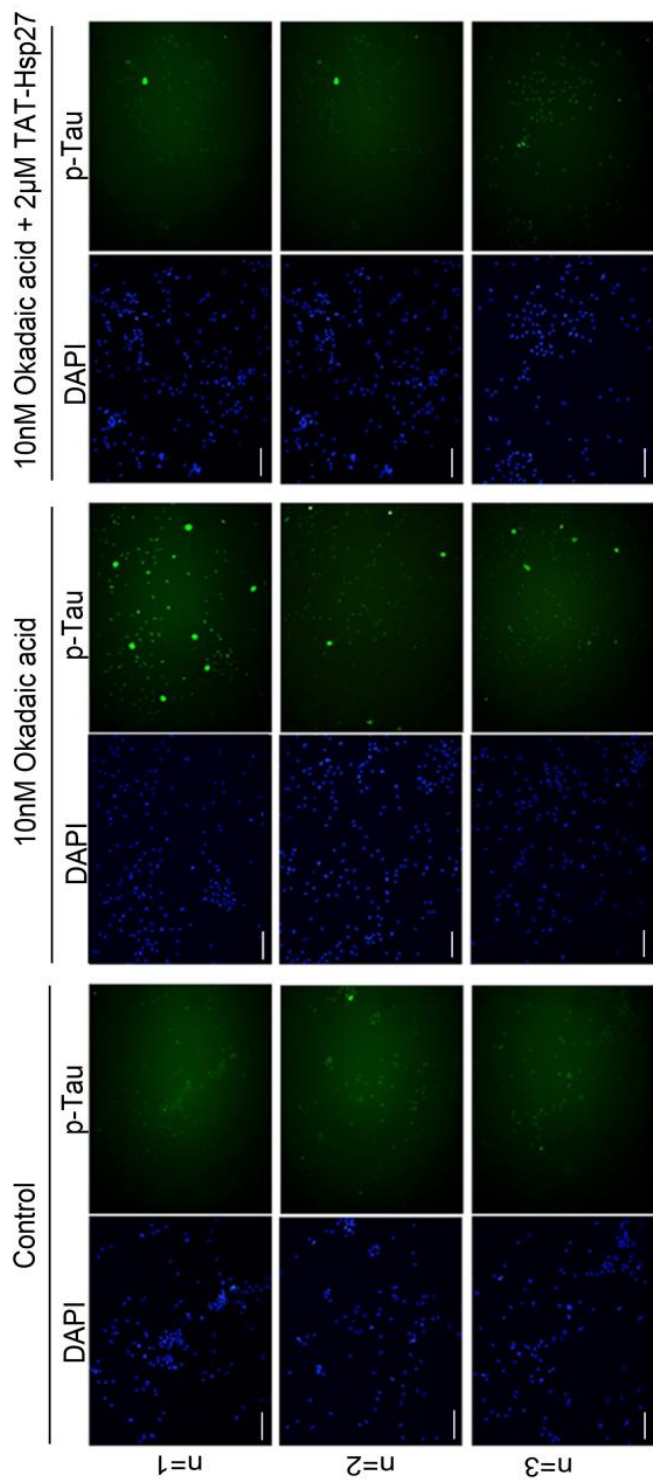
Figure 4. Representative immunofluorescence images. SH-SY5Y cells were treated or not with 10 nM okadaic acid alone or 2 μM Tat-Hsp27 (2 h) followed by 10 nM okadaic acid (14 h). All cells were fixed and stained with primary antibodies against phosphorylated tau and the corresponding fluorescent secondary antibody. Green (FITC) indicates phosphorylated tau. Scale bar: 100 μm.
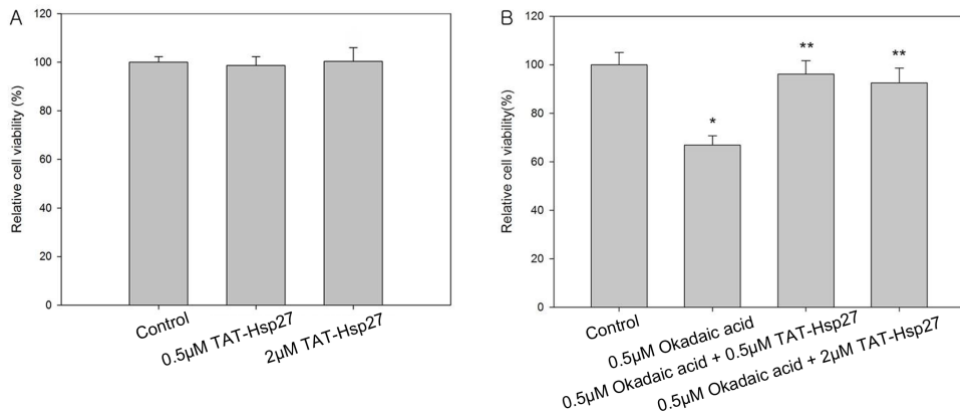
Figure 5. Cytotoxicity assay using the Cell Counting Kit-8. SH-SY5Y cells were transduced with 0.5 μM or 2 μM Tat-Hsp27 alone (A) or prior to treatment with 0.5 µM okadaic acid (B). The transduction of Tat-Hsp27 alone does not affect the cell viability. *p < 0.05 compared with the control. **p < 0.01 compared with okadaic acid alone. The data are presented as the mean ± SD (n = 7).
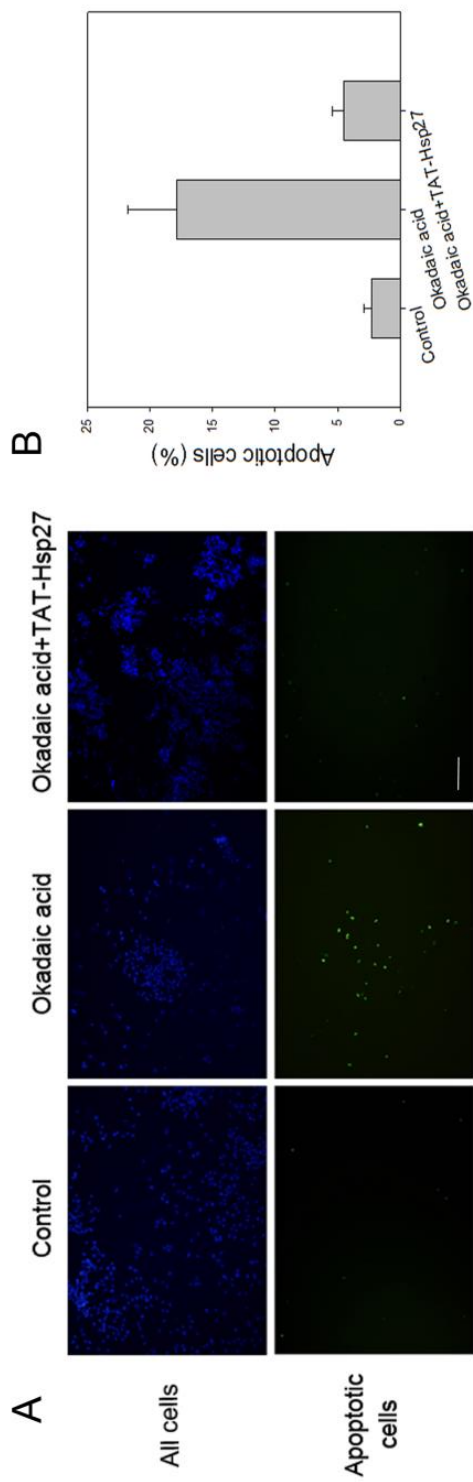
Figure 1. Tat-Hsp27 inhibits apoptotic cell death induced by okadaic acid. The TUNEL assay was performed to determine the extent of programmed cell death. SH-SY5Y cells were treated with 10 nM okadaic acid alone or following pretreatment with 2 μM Tat-Hsp27. (A) Representative images of the TUNEL assay. Scale bar: 100 μm. (B) Quantitation of the percentage of TUNEL-positive cells indicated that apoptosis induced by okadaic acid was decreased in cells pretreated with Tat-Hsp27. The data represent the mean ± SD (n = 3).

8. Abstract in Korean (국문초록)

알츠하이머는 뇌 기능을 점차 상실하게 되는 노화관련 신경퇴행성 질병이다. 세포 골격 유지에 관여하는 타우 단백질이 과인산화되면서 불용해성 섬유인 세포내 신경 원섬유엉킴 (neurofibrillary tangles, NFTs)가 형성되는데, NFTs 는 알츠하이머를 결정짓는 병리학적 증상으로 알려져 있다. 따라서 타우 단백질의 과인산화를 조절하는 것은 알츠 하이머의 치료에 있어 중요하고 기본적인 접근법이라 할 수 있다.

열 충격 단백질 (Hsp)은 비정상적인 접힘을 이루는 단 백질이 정상적인 접힘을 할 수 있도록 돕는 역할을 한다. 이와 관련하여 신경퇴행성과 열 충격 단백질과의 관 계가 최근 연구되고 있지만 아직 알츠하이머 치료제로 써의 연구는 미비한 상황이다. 본 연구에서는 Hsp27을 이 용하여 알츠하이머 유발 모델에서의 치료효과를 입증하였다.

Hsp27을 세포 내로 효과적으로 전달하기 위하여 단백 질 전달 도메인 (protein transduction domain, PTD) Tat을 열 충격 단백질 27에 연결하였다 (Tat-Hsp27). 타우 단백질의

과인산화를 유도한 후 Tat-Hsp27을 처리하여 과인산화된 타우 단백질의 감소를 확인하였고 이로 인해 세포 생존률 또한 크게 향상되는 것을 확인하였다.

주요어: 타우 단백질, 과인산화, 알츠하이머 병, 열 충격 단백질 27, 단백질 전달 도메인

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