Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 346 (2006) 1–6
BBRC
www.elsevier.com/locate/ybbrc

A novel therapeutic approach to 6-OHDA-induced Parkinson's disease in rats via supplementation of PTD-conjugated tyrosine hydroxylase

Shao Ping Wu [a,b], Ai Ling Fu [a], Yu Xia Wang [a], Lei Ping Yu [c], Pei Yuan Jia [a], Qian Li [a], Guo Zhang Jin [c], Man Ji Sun [a,*]

[a] *Department of Biochemistry and Pharmacology, Beijing Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Tai Ping Road, 27, Beijing 100850, China*
[b] *Beijing Centers for Diseases Prevention and Control, Beijing 100013, China*
[c] *Department of Pharmacology, State Key Laboratory of Drug Research, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China*

Received 7 March 2006
Available online 2 May 2006

Abstract

The present study aimed to evaluate whether the protein transduction domain (PTD)-conjugated human tyrosine hydroxylase (TH) fusion protein was effective on the 6-hydroxydopamine (6-OHDA)-induced Parkinson's disease (PD) model rats. An expression vector pET-PTD-TH harbouring the PTD-TH gene was constructed and transformed to the *Escherichia coli* BL21 cells for expression. The expressed recombinant PTD-TH with a molecular weight of 61 kD was successfully transduced (1 μM) into the dopaminergic SH-sy5y human neuroblastoma cells in vitro and visualized by immunohistochemical assay. An in vivo experiment in rats showed that the iv administered PTD-TH protein (8 mg/kg) permeated across the blood–brain barrier, penetrated into the striatum and midbrain, and peaked at 5–8 h after the injection. The behavioral effects of PTD-TH on the apomorphine-induced rotations in the PD model rats 8 weeks after the 6-OHDA lesion showed that a single bolus of PTD-TH (8 mg/kg) iv injection caused a decrement of 60% of the contralateral turns on day 1 and 40% on days 5–17. The results imply that iv delivery of PTD-TH is therapeutically effective on the 6-OHDA-induced PD in rats, the PTD-mediated human TH treatment opening a promising therapeutic direction in treatment of PD.

Keywords: Tyrosine hydroxylase; Protein transduction domain; 6-Hydroxydopamine; Parkinson's disease

Parkinson's disease (PD) was originally described by James Parkinson in 1817. PD is one of the most common neurodegenerative disorders that strikes 1–2% of persons older than 40 years of age [1], and affects about 4 million people worldwide [2]. PD is a progressive, age-associated, neurodegenerative disease characterized by bradykinesia, resting tremor, rigidity, and gait disturbance. The main anatomical feature of PD is the decrease in number of neuromelanin-containing neurons located in the midbrain substantia nigra pars compacta (SNpc). These dopaminergic (DAergic) neurons project to the striatum as well as a number of other subcortical regions [3]. Degeneration of the DAergic neurons located in the SNpc and a subsequent loss of DAergic nerve terminals in the striatum are responsible for most of the movement disturbances [4]. In idiopathic PD, the symptoms become apparent when about 70% of the striatal DA and about 50% of the nigral DAergic neurons are lost [5]. Since the tyrosine hydroxylase (tyrosine 3-monooxygenase, EC 1.14.16.2, TH) catalyzes the conversion of L-tyrosine to DOPA. Currently, the strategy for gene therapy of PD involving local production of dopamine in the striatum achieved by inducing the expression of TH, the rate-limiting enzymes in the biosynthetic pathway for dopamine [6], was supposed and resulted in biologically significant functional recovery [7–9]. The aim of present study is to assess whether it is

* Corresponding author.
E-mail address: Sunmj@nic.bmi.ac.cn (M.J. Sun).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.03.247

possible to improve the performance in PD model rats via supplementation with exogenous TH protein.

The native human TH is a monooxygenase existing as a homotetramer, each subunit having 497 aa with a molecular weight of 55 kD, and containing one single catalytic site. The catecholamine biosynthetic reaction catalyzed by TH requires the natural cofactor, (6*R*)-L-erythro-tetrahydrobiopterin, and the ferrous ion [10]. The human TH gene maps to the short arm chromosome region 11P15.5. The sequences of the human TH deduced from the nucleotide sequences of the cDNAs reveal four distinguishable types of isozymes producing via the alternative post-transcriptional splicing of the mRNA. Among them, the TH1 has the highest specific enzymatic activity, 2.5- to 3-fold higher than those of the rest three isozymes. Here in our experiment, the human TH1 gene was used in construction of the recombinant plasmid for the production of the PTD fusion protein.

The delivery of therapeutic protein into brain across the blood–brain barrier is severely limited by the size and biochemical properties of the proteins. At present, an approach to deliver the therapeutic peptides or proteins to brain is the application of fusion proteins linked to so-called protein transduction domains (PTDs). The basic domain (47–57, YGRKKRRQRRR) of the human immunodeficiency virus (HIV-1), one kind of PTD, has been shown to deliver various peptides or proteins into many different types of cells and tissues [11–13], Here in this paper, a recombinant PTD-TH fusion protein was delivered into the brain of the PD-model rats for the therapeutic purpose.

Materials and methods

Construction of pET-PTD-TH plasmid. The TH gene was amplified by PCR from the plasmid pET3c-TH bearing the human TH gene, using two primers: P1, GCAGAATTCATGCCCACCCCCGACG; P2, GCTGTCGACCTAGCCAATGGCACTCA (the *Eco*RI and *Sal*I sites were underlined). The PCR was performed using *Pfu* DNA polymerase at 94 °C for 40 s, 56 °C for 30 s, and 72 °C for 2 min for 30 cycles. The PCR product was purified using a Clontech Chromospin column, cut with *Eco*RI and *Sal*I, and cloned into pET28a to construct the pET-TH plasmid. The sense chain 5′ GATCCTATGGTCGTAAAAAGCGACGCCAACGTAGAC GTG and the antisense chain: 5′ AATTCACGTCTACGTTGGCGTC GCTTTTTACGACCATAG (*Bam*HI and *Eco*RI restriction sites were underlined) encoding the 11 amino acids of the HIV-1 Tat were then ligated into *Bam*HI and *Eco*RI-digested pET-TH to construct the pET-PTD-TH plasmid. The nucleotide sequences of the plasmids were analyzed for correctness by Bioasia (Shanghai, China).

Expression and purification of PTD-TH fusion protein. The fusion proteins were expressed and induced by IPTG in *Escherichia. coli* BL21 transformed with the plasmid pET-PTD-TH or the plasmid pET-TH without the PTD gene as the control. The bacterial pellets were washed with PBS, resuspended in PBS, and supersonicated on ice for 30 cycles (10 s on and 20 s off). The sonicates were centrifuged at 16,000*g* for 15 min at 4 °C. The pellet was solubilized in buffer A (8 M urea, 100 mM NaCl, and 20 mM Tris–HCl, pH 8.0), added imidazole to a concentration of 10 mM, and applied onto the pre-equilibrated Ni–NTA column at room temperature. The column was then washed with 10 mM imidazole in buffer A, and the enriched fusion protein was eluted from the Ni–NTA column by 500 mM imidazole. The purified fractions were pooled and dialyzed in buffer B (0.1 mM GSH, 0.01 mM GSSG, 100 mM NaCl, and 20 mM Tris–HCl, pH 8.0) to remove the urea, and then dialyzed against PBS. The purified fusion protein was stored at −20 °C in aliquots. The protein concentrations were measured according to Bradford [14] using the bovine serum albumin as the standard.

Cell culture and treatment. SH-sy5y cells were cultured in the RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin under conditions of 5% CO_2–95% air at 37 °C. Cells were plated on the chamber slides (10^4 cells/cm^2) and cultured for 48 h prior to treatments with 1 μM TH or PTD-TH. In the control group, the PBS was used instead of the TH and PTD-TH. Two hours after exposure, the layered cells were rinsed thrice with PBS and subjected to immunohistochemical analysis.

Animals. Male Sprague–Dawley rats (220 ± 10 g, Grade II, Shanghai Experimental Animal Center, Certificate No. 005 conferred by Animal Management Committee, Chinese Academy of Sciences) were housed two per cage at 20–22 °C on a 12-h light–dark cycle with food and water *ad libitum*.

Immunohistochemical analysis. The biotin–streptavidin–HRP method was used for the immunohistochemistry. Rats were injected 8 mg/kg PTD-TH through the caudal vein. Four hours later, the rats were anesthetized and fixed by intracardical perfusion of 4% paraformaldehyde in PBS (pH 7.4). The brains were removed and kept at −20 °C ready for use. After blocking the endogenous peroxidase with 3% hydrogen peroxide for 15 min, the tissue slices (30 μm) were incubated with the non-specific goat serum for 2 h at room temperature, and then incubated with the rabbit anti-rat TH polyclonal antibody (1:2000 diluted in PBS), the biotin-labelled goat anti-rabbit IgG antibody, and the horseradish peroxidase-labelled streptavidin, and finally reacted with 0.05% diaminobenzidine (DAB) and 0.001% hydrogen peroxide. The images were examined under a Zeiss microscope.

Western blotting. The midbrain and striatum were dissected and homogenized, respectively, in the lysis buffer (20 mM Tris–HCl, 5 mM EDTA, 1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 10 mM Chaps, and 1% Triton X-100). The centrifuged supernatants were subjected to the SDS–PAGE and then transferred onto the polyvinyl difluoride membranes. The membranes were blocked in the blocking solution (5% skimmed milk, 10 mM Tris–HCl, 100 mM NaCl, and 0.1% Tween 20), and immersed with the rabbit anti-rat TH polyclonal antibody or the mouse anti-GAPDH monoclonal antibody (diluted 1:200 in 0.1% Tween 20–0.1 M Tris-buffered saline), followed by the horseradish peroxidase-conjugated secondary antibodies, visualized by the enhanced chemiluminescence (ECL), and then exposed to the ECL-Hyperfilm.

Unilateral medial forebrain bundle (MFB) lesion. Rats were treated with 20 mg/kg desmethylimipramine 20 min before anesthesia with choral hydrate (400 mg/kg) and then placed in a stereotaxic frame (David Kopf, Tujunga, CA). The skull was exposed and a burr hole was drilled to introduce a syringe for a single bolus injection of the 6-OHDA solution 45 min after desmethylimipramine treatment in order to minimize variability due to degradation of the toxin. The 6-OHDA solution was freshly made, kept on ice, and protected from exposure to the light. The 6-OHDA (8 μg/4μl dissolved in 0.9% saline with 0.1 mg/ml ascorbic acid, pH 5.0) was injected into MFB (coordination: AP −4.0 mm, ML 1.65 mm, DV −8.0 mm according to the rat atlas of Paxinos and Watson [15]) at a rate of 1 μl/min. The needle was left in the place for additional 5 min to allow complete diffusion of the medium and minimize the diffusion of toxin along the needle track. The incised skin was then sutured. The animals were ip injected 4 mg/kg ketofen as analgesic and were allowed to recover before returning to the animal housing facilities.

Behavioral testing. Four weeks after 6-OHDA injection, rats were tested with apomorphine (0.5 mg/kg, ip) to evaluate the behavioral effect of the nigrostriatal lesion (turning behavior). The number of full rotations performed by the animal was registered for 30 min (starting 1 min after apomorphine injection), using a video camera system (Jiliang, Shanghai, China). Rotational response was expressed in terms of the number of the net full turns (360°) per minute.

Statistical analysis. The data are expressed as means ± SEM. Differences between groups were compared by using the Student's *t* test. Values of $P < 0.05$ were considered to indicate statistical significance.

Results

Construction of the expression vector of the PTD-TH fusion protein gene

In an effort to deliver the exogenous protein, we adopted a protein transduction approach to introduce the TH into the brain. To produce the cell-permeable TH fusion protein, an expression vector bearing the PTD-TH gene was constructed (Fig. 1). A plasmid without PTD gene was constructed as the control. The expressed PTD-TH fusion protein showed a 61 kD band (Fig. 2) did not differ very much from that of the control, since the PTD has only a small molecular mass of 1.2 kD. The Western blot using the rabbit anti-rat TH polyclonal antibody showed the bands corresponding to the purified PTD-TH and TH (Fig. 3).

Transduction of PTD-TH fusion protein into cells

The PTD-TH fusion protein was added to the dopaminergic SH-sy5y human neuroblastoma cells [16] at concentration of 1 μM. One hour later, the PTD-TH permeated into the cells was subjected to the immunohistochemical analysis. As shown in Fig. 4C, the cells dealt with PTD-TH showed intensive staining in the cytoplasm of the SH-sy5y cells, whereas the PBS control (Fig. 4A) and

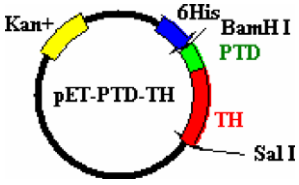

Fig. 1. Schematic profile of the PTD-TH expression vector.

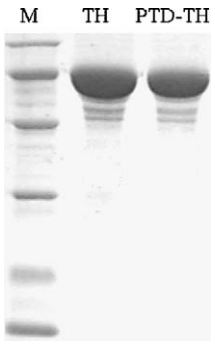

Fig. 2. SDS–PAGE of the purified TH and PTD-TH proteins expressed in BL21 (DE3). Coomassie brilliant blue staining. M, molecular markers (97.4, 66.2, 43.0, 31.0, 20.1, 14.4 kD).

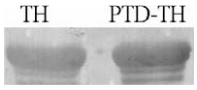

Fig. 3. Western blot of TH and PTD-TH expressed in BL21 (DE3).

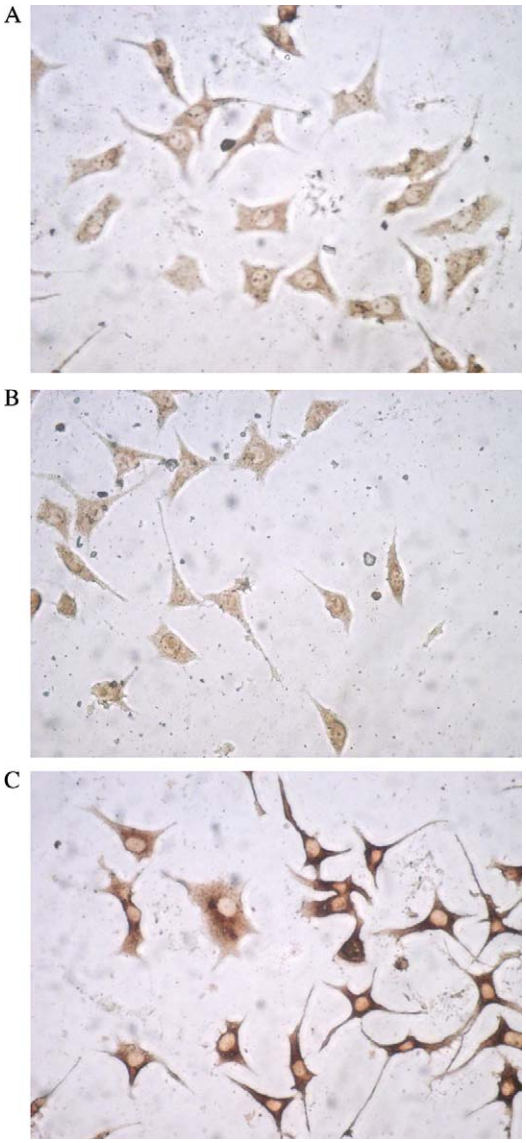

Fig. 4. Immunohistochemical analysis of TH in the human neuroblastoma cells SH-sy5y. SH-sy5y cells were incubated with 1 μM TH or PTD-TH for 1 h. Immunohistochemical assay was performed using biotin–streptavidin–HRP method with rabbit anti-TH polyclonal antibody and visualized with DAB. (A) Control, PBS. (B) TH without PTD. (C) PTD-TH.

TH without PTD did not (Fig. 4B). It implies that the human TH has well penetrated into the SH-sy5y cells by virtue of the action of the PTD.

Transduction of PTD-TH fusion protein into brain

To determine whether the purified PTD-TH intravenously injected could pass through the blood–brain barrier, the TH protein in the brains of rats was detected using the immunohistochemical assay 4 h after the injection of PTD-TH or TH (8 mg/kg). The results showed that intensive staining was observed in the slices of brain after injection of PTD-TH (Fig. 5B) in sharp contrast with that of the control mice injected TH without bearing PTD (Fig. 5A).

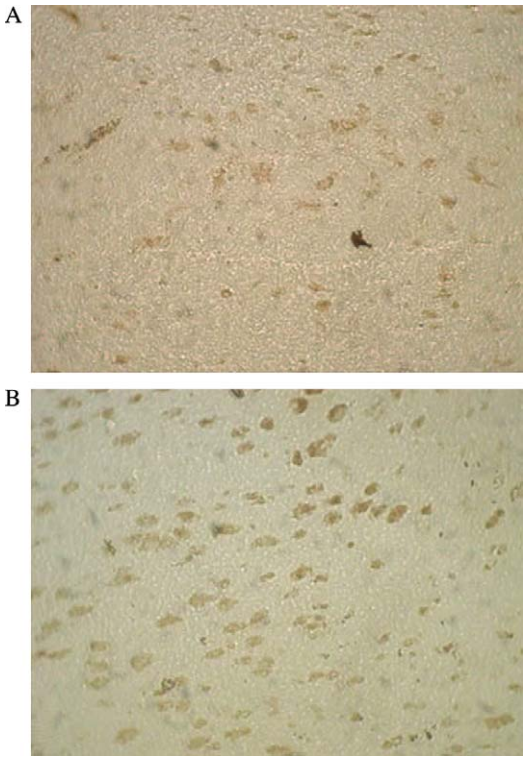

Fig. 5. Immunohistochemical analysis of TH in the brain after intravenous administration of PTD-TH or TH. Immunohistochemistry with polyclonal rabbit anti-TH antibody and visualized with DAB. (A) TH without PTD. (B) PTD-TH.

Time course of transduction of PTD-TH fusion protein into rat brains

Rats were administered 8 mg/kg PTD-TH through the caudal vein injection. The TH protein in the striatum and midbrain was analyzed via Western blot at 0, 1, 2, 5, 8, 12, 23, and 31 h after the injection. The results showed that the PTD-TH protein has penetrated into the striatum and midbrain, increased along with time, peaked at 5–8 h, and then declined to the intrinsic level gradually (Fig. 6A–C).

Effect of purified PTD-TH fusion protein on apomorphine-induced rotations in 6-OHDA impaired rats

To evaluate the behavioral effect of the purified PTD-TH fusion protein on apomorphine-induced rotations in 6-OHDA impaired rats, a single bolus of 8 mg/kg PTD-TH was iv injected into each rat 8 weeks after the 6-OHDA lesion. In the control rats, equal volumes of saline were iv injected instead of the PTD-TH. Apomorphine-induced rotations were tested at 1, 5, and 17 days after iv administration of the PTD-TH protein. The time–effect curve showed that the rotation rates of the control rats were kept at a high level during the 18 days of observation, whereas that of the rats iv injected with PTD-TH decreased 60% on day 1 and 40% on days 5–17 (Fig. 7).

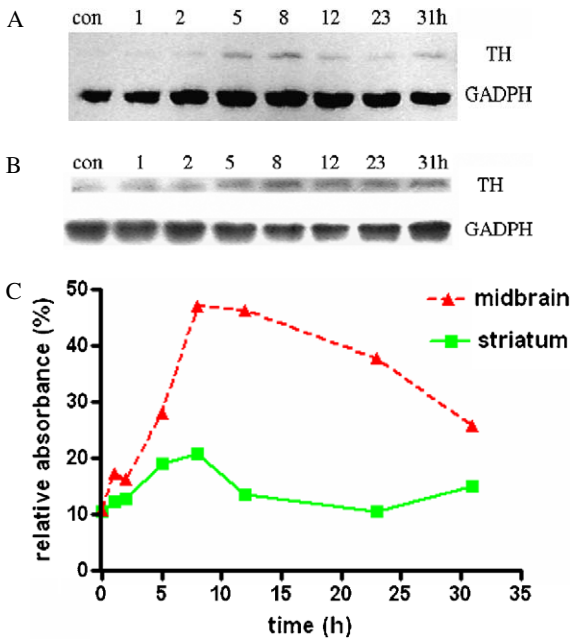

Fig. 6. Western blot of transduced PTD-TH fusion proteins in rat striatum and midbrain. Rats were iv administered 8 mg/kg PTD-TH, the striatum and midbrain were detected at 1, 2, 5, 8, 12, 23, and 31 h after iv injection. Rabbit anti-TH polyclonal antibody or mouse anti-GADPH monoclonal antibody was used, and the slices were visualized with ECL. (A) Striatum, (B) midbrain, (C) time course plot. Relative absorbance = absorbance of TH/absorbance of GADPH × 100%.

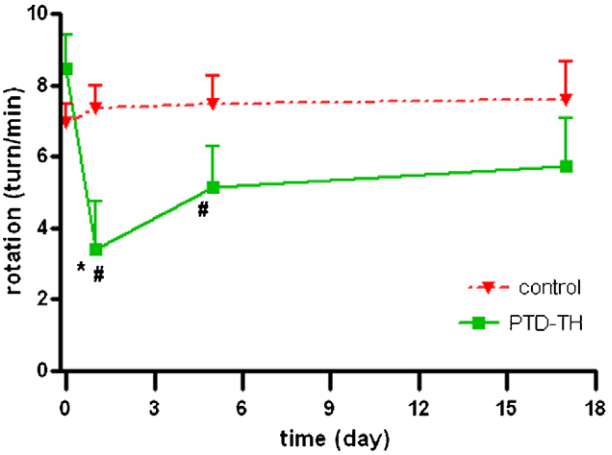

Fig. 7. Behavioral test of rats iv injected with PTD-TH. PTD-TH, iv 8 mg/kg, 8 weeks after 6-OHDA impairment (mean ± SEM, $n = 8$); control, iv equal volumes of saline (mean ± SEM, $n = 6$). At days 1, 5, 17 after the injection rats were challenged with apomorphine, and the circling behavior was monitored for 30 min in terms of net contralateral turns/min. $^{*}P < 0.05$ vs controls, $^{\#}P < 0.05$ vs the zero time (Student's t test).

Discussion

The delivery of therapeutic proteins into tissues and across the blood–brain barrier (BBB) is severely limited by the size and biochemical properties of the respective proteins. The BBB is the bottleneck in brain drug development and is the most important factor limiting the future

growth of neurotherapeutics [17], the protein transduction domains derived from the TAT protein of HIV-1, the antennapedia homeodomain of drosophila (AntpHD) or the simplex herpes virus VP22 protein [18] are capable of crossing the BBB in the delivery of many fused peptides and proteins into the brain [19,20]. The present study also successfully showed a progressive translocation of PTD-TH proteins into the rat brain tissue.

The TAT motif can functionally be dissected into two parts: GRKKR acts as a potential nuclear localization signal (NLS), whereas RRR appears to be very critical for protein translocation [21]. The TAT-p53 conjugate was efficiently delivered to both the cytoplasm and the nucleus of cells [13], the TAT-GFP expressed in mammalian cells was located predominantly in the nucleus [22]. Here, we showed in our results that the PTD-TH existed mainly in the cytoplasm and little in the nucleus, similar to the TAT-5(6)-carboxyfluorescein which was delivered mainly in the cytoplasm of the MDCK cells [23]. It seems that the subcellular localizations of the PTD fusion protein depended on the nature of the fusion protein moiety rather than PTD [12,22,24].

PD is characterized by a trias of cardinal symptoms, namely bradykinesia, resting tremor, and rigidity. A relatively specific pathological feature is the degeneration of the dopaminergic neurons located in the substantia nigra pars compacta and leading to a reduction of the striatal DA content. TH catalyzes the initial and rate-limiting step in the biosynthetic pathway of catecholamines, including dopamine, noradrenaline, and adrenaline. Thus, we conceived that an alternative therapy via supplementation of PTD-TH would be a potential remedy for PD. This supposal is attractive not only for theoretical interests but also important for practical application. In this paper, the effectiveness of the transduction of PTD-TH in the therapy of 6-OHDA-induced Parkinson's disease has been successively verified in rats. It has been documented that human produces four different forms of TH mRNA via alternative splicing from a single gene. The isozyme TH1 has the highest homospecific activity (activity per enzyme protein), the values for the other isoenzymes ranging from 30% to 40% [25]. Therefore, the human TH1 isozyme was chosen to construct the PTD-conjugate for treatment PD in the model rats.

In conclusion, the present study shows that iv delivery of PTD-TH is effective on the neuronal injury induced by 6-OHDA. The PTD-mediated human TH treatment opens a promising therapeutic direction in the therapy of Parkinson's diseases.

Acknowledgments

We are grateful to the gift of the plasmid pET3c-TH from Professor Toshiharu Nagatsu and Professor Akira Ota of Fujita Health University, Japan.

References

[1] C.M. Tanner, S.M. Goldman, Epidemiology of Parkinson's disease, Neurol. Clin. 14 (1996) 317–335.
[2] D.M. Bushnell, M.L. Martin, Quality of life and Parkinson's disease: translation and validation of the US Parkinson's Disease Questionnaire (PDQ-39), Qual. Life Res. 8 (1999) 345–350.
[3] T.N. Chase, J.D. Oh, P.J. Blanchet, Neostriatal mechanisms in Parkinson's disease, Neurology 51 (1998) S30–S35.
[4] T. Nagatsu, M. Mogi, H. Ichinose, A. Togari, Changes in cytokines and neurotrophins in Parkinson's disease, J. Neural. Transm. Suppl. (2000) 277–290.
[5] S.B. Dunnett, A. Bjorklund, Prospects for new restorative and neuroprotective treatments in Parkinson's disease, Nature 399 (1999) A32–A39.
[6] T. Nagatsu, M. Levitt, S. Udenfriend, Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis, J. Biol. Chem. 239 (1964) 2910–2917.
[7] L. Lu, C. Zhao, Y. Liu, X. Sun, C. Duan, M. Ji, H. Zhao, Q. Xu, H. Yang, Therapeutic benefit of TH-engineered mesenchymal stem cells for Parkinson's disease, Brain Res. Brain Res. Protoc. 15 (2005) 46–51.
[8] W.M. Pardridge, Tyrosine hydroxylase replacement in experimental Parkinson's disease with transvascular gene therapy, NeuroRx. 2 (2005) 129–138.
[9] L. Cao, Y.C. Zhao, Z.H. Jiang, D.H. Xu, Z.G. Liu, S.D. Chen, X.Y. Liu, Z.C. Zheng, Long-term phenotypic correction of rodent hemiparkinsonism by gene therapy using genetically modified myoblasts, Gene. Ther. 7 (2000) 445–449.
[10] A.R. Brenneman, S. Kaufman, The role of tetra-hydropteridines in the enzymatic conversion of tyrosine to 3,4-dihydroxyphenylalanine, Biochem. Biophys. Res. Commun. 17, 177–183.
[11] S.R. Schwarze, K.A. Hruska, S.F. Dowdy, Protein transduction: unrestricted delivery into all cells? Trends Cell Biol. 10 (2000) 290–295.
[12] A.L. Fu, S.J. Huang, M.J. Sun, Complementary remedy of aged-related learning and memory deficits via exogenous choline acetyltransferase, Biochem. Biophys. Res. Commun. 336 (2005) 268–273.
[13] J. Ryu, H.J. Lee, K.A. Kim, J.Y. Lee, K.S. Lee, J. Park, S.Y. Choi, Intracellular delivery of p53 fused to the basic domain of HIV-1 Tat, Mol. Cells 17 (2004) 353–359.
[14] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.
[15] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, San Diego, 1998.
[16] T. Tanaka, K. Iqbal, E. Trenkner, D.J. Liu, I. Grundke-Iqbal, Abnormally phosphorylated tau in SY5Y human neuroblastoma cells, FEBS Lett. 360 (1995) 5–9.
[17] W.M. Pardridge, The blood–brain barrier: bottleneck in brain drug development, Neurosci. Res. 2 (2005) 3–14.
[18] J.A. Leifert, J.L. Whitton, "Translocatory proteins" and "protein transduction domains": a critical analysis of their biological effects and the underlying mechanisms, Mol. Ther. 8 (2003) 13–20.
[19] A.L. Fu, Q. Li, Z.H. Dong, S.J. Huang, Y.X. Wang, M.J. Sun, Alternative therapy of Alzheimer's disease via supplementation with choline acetyltransferase, Neurosci. Lett. 368 (2004) 258–262.
[20] U. Kilic, E. Kilic, G.P. Dietz, M. Bahr, Intravenous TAT-GDNF is protective after focal cerebral ischemia in mice, Stroke 34 (2003) 1304–1310.
[21] E. Vives, P. Brodin, B. Lebleu, A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus, J. Biol. Chem. 272 (1997) 16010–16017.

[22] Y. Yang, J. Ma, Z. Song, M. Wu, HIV-1 TAT-mediated protein transduction and subcellular localization using novel expression vectors, FEBS Lett. 532 (2002) 36–44.
[23] R. Trehin, U. Krauss, R. Muff, M. Meinecke, A.G. Beck-Sickinger, H.P. Merkle, Cellular internalization of human calcitonin derived peptides in MDCK monolayers: a comparative study with Tat (47–57) and penetratin (43–58), Pharm. Res. 21 (2004) 33–42.
[24] G. Cao, W. Pei, H. Ge, Q. Liang, Y. Luo, F.R. Sharp, A. Lu, R. Ran, S.H. Graham, J. Chen, In vivo delivery of a Bcl-xL fusion protein containing the TAT protein transduction domain protects against ischemic brain injury and neuronal apoptosis, J. Neurosci. 22 (2002) 5423–5431.
[25] T. Nagatsu, The human tyrosine hydroxylase gene, Cell Mol. Neurobiol. 9 (1989) 313–321.