The expression and significance of tyrosine hydroxylase in the brain tissue of Parkinson's disease rats

YUAN CHEN, YAJUN LIAN, YUNQING MA, CHUANJIE WU, YAKE ZHENG and NANCHANG XIE

Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, P.R. China

Received April 18, 2017; Accepted September 6, 2017

DOI: 10.3892/etm.2017.5124

Abstract. The expression and significance of tyrosine hydroxylase (TH) in brain tissue of rats with Parkinson's disease (PD) were explored and analyzed. A total of 120 clean-grade and healthy adult Wistar rats weighing 180-240 g were randomly divided equally into four groups according to the random number table method. Rats were sacrificed before and after the model establishment for 3, 6 or 8 weeks. The number of revolutions in rats was observed and the relative expression of TH mRNA in brain tissue was measured. The relative expression of TH mRNA in left and right brain tissue of normal rats was respectively 1.039±0.112 and 0.956±0.120. There was no significant difference in the expression of TH mRNA in either side of the brain tissue ($p>0.05$). With the extension of modeling time, the success rate of model establishment was significantly improved ($p<0.05$). After the model establishment for 6 weeks, the success rate peaked and there was no significant difference with 8 weeks ($p>0.05$). The relative expression of TH mRNA in the right brain was 0.053±0.082, which was significantly lower than that in the left brain tissue with 1.056±0.094 ($p<0.05$). After 6 weeks, the relative expression of TH mRNA in the left and right brain of PD rats was respectively 1.004±0.034 and 0.316±0.012, the expression of TH mRNA in right brain tissue was also significantly lower than that in the left ($p<0.05$). Similarly, after 8 weeks of the model establishment, the relative expression of TH mRNA in the right brain was significantly lower than that in the left brain tissue, with 0.395±0.041 and 1.021±0.057 ($p<0.05$). Compared with the control group, the expression of TH mRNA in both sides of the brain tissue decreased, and the expression of TH mRNA in the injured brain tissue was significantly lower than that in normal rats ($p<0.05$). The expression of TH mRNA in brain tissue of PD rats was lower than that in normal brain tissue, which may be related to the occurrence and development of PD.

Correspondence to: Dr Yajun Lian, Department of Neurology, The First Affiliated Hospital of Zhengzhou University, 1 Jianshedong Road, Zhengzhou, Henan 450052, P.R. China
E-mail: dryajunlian@163.com

Key words: Parkinson's disease, tyrosine hydroxylase, animal model

Introduction

Parkinson's disease (PD) is a common chronic degenerative disease of the nervous system, and the incidence in people is mainly in middle-age. The motor neuron disorders are the main lesion of PD, manifested as muscle tremor, mobility and coordination capacity decrease (1,2). PD not only affects the health of patients, but also seriously affects the quality of patient's life (3). Currently the pathogenesis of PD is not clear, but the main point now is that it is related to the degeneration of dopaminergic neurons. With the progress of the disease, neuronal lesion results in the gradual reduction of dopamine synthesis, leading to abnormal discharge in cerebral cortex. Tyrosine hydroxylase (TH) is a rate-limiting enzyme for dopamine synthesis, which plays an important role in the synthesis of dopamine and may be related to the development of PD (4,5). Some studies shown that intervening in the expression and synthesis of TH could effectively improve the neurological symptoms of PD rats (5,6). In this study, we investigated the expression of TH in brain tissue, in order to explore the expression level and significance of TH in PD.

Materials and methods

Animals. Experiments were performed using healthy adult Wistar rats weighing 180-240 g, provided by our College Animal Center. Rats were kept in the animal house under constant temperature (18-25˚C) and humidity (60-70%) on natural light with the C060 sterilized commercial feed and free water intake, with 12 h before fasting. The study was approved by the Ethics Committee of Zhengzhou University.

Experimental design. Before modeling, rats were examined repeatedly to observe whether they had rotational behavior and to ensure that all rats were healthy.

Modeling method. Rats were fixed and anesthetized with injection of 2% pentobarbital sodium (0.2 ml/100 g). Then the hair was removed of the rat head and fixed in the stereotaxic device, with the ear canal and the incisors fixed, it is important to note that the door hooks plane was 2.4 mm lower than the external auditory canal connection. The skin around the incision was disinfected, and the middle incision was used to separate the rat subcutaneous tissue layer and layer to expose the rat skull. After removal of the skull, the meninges was

Table I. Induction time of rats with different induction times.

Group	N	Modeling situation (n, %)			
		2 weeks	3 weeks	6 weeks	8 weeks
No. of >7 r/min rats	120	78	68	51	26
Induction success rate		78/120 (65.0%)	68/90 (75.5%)[a]	51/60 (85%)[a]	26/30 (86.7%)[a]

[a]p<0.05 vs. the group 2 weeks after operation.

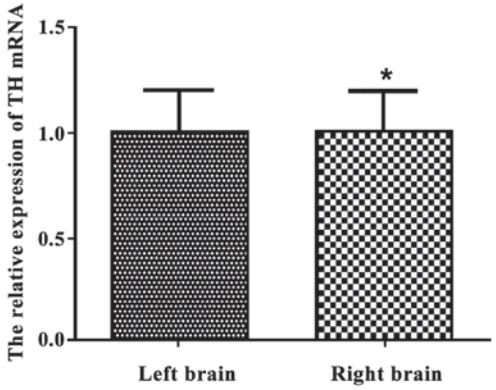

Figure 1. The relative expression of TH mRNA in normal brain tissues. *p>0.05.

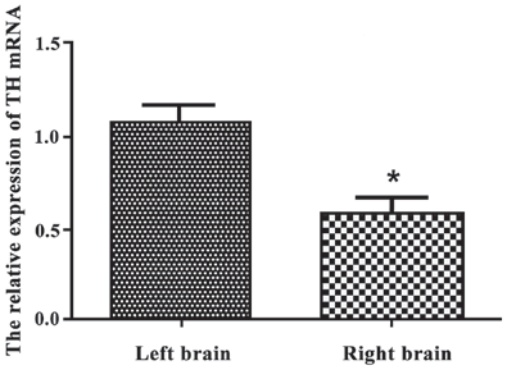

Figure 2. The relative expression of TH mRNA in brain tissues of PD model rats for 3 weeks after operation. *p<0.05.

removed followed by wiping with 30% hydrogen peroxide, using the front flotation points as benchmark and the injection point positioning and methods refer to the stereotactic parameters for modeling PD introduced by He (7). After the successful injection, the wound was sutured and disinfected regularly, with intraperitoneal injection of penicillin for one week continuous infection.

Evaluation of success criteria for rat modeling. After subcutaneous injection of apomorphine (APO) in the rat neck with the dose of 0.05 mg/kg, induced rat rotation to the rotational behavior, the rotations were counted over a period of 30 min in the rat PD model. All rats were randomly divided equally into four groups (A-D) according to the random number table method. Rats were sacrificed before and after the model establishment for 3, 6 or 8 weeks. The number of revolutions in rats was counted and the relative expression of TH mRNA in brain tissue was measured by qPCR.

Observation and evaluation of indicators. After modeling operation 3, 6 and 8 weeks, the revolutions was counted and recorded to evaluate the success of modeling. Modeling success indicators: Number of revolutions >7 r/min can be determined as successful modeling. The relative expression level of TH mRNA in both sides of brain tissue in each group was detected.

Statistical analysis. The statistical analysis was performed with SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) and the obtained data are in normal distribution. Measurement data are expressed as mean ± SD and the t-test was performed. The non-parametric data were analyzed by rank sum test. The data were calculated by Chi-square test and repeated variance analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

The relative expression level of TH mRNA in both sides of brain tissues in normal rats. As shown in Fig. 1, the relative expression of TH mRNA was respectively 1.039±0.112 and 0.956±0.120 in left and right brain tissues of normal rats. There was no significant difference (p>0.05) between the two sides of brain tissues in normal rats.

The success rates in each group of modeling. With the extension of modeling time, the success rate of modeling was significantly increased, and the success rate of induction was close to the peak value at 6 weeks after operation. The success rate was not significantly increased after (p>0.05), as shown in Table I.

The relative expression of TH mRNA in the left and right brain tissue of PD rats after operation for 3 weeks. For 3 weeks after operation, the relative expression of TH mRNA in the left brain tissue of PD rats was more than the right brain tissue, with 1.056±0.094 and 0.053±0.082, respectively. There was statistically significant deference between the sides of brain tissues (p<0.05), as shown in Fig. 2.

The relative expression of TH mRNA in the left and right brain tissue of PD rats after operation for 6 weeks. As shown in Fig. 3, the relative expression of TH mRNA in the left brain tissue of PD rats was more than that in the right brain tissue

Table II. Comparison of the relative expression of TH mRNA (mean ± SD).

Group	Relative expression of TH mRNA			
	Preoperative (n=30)	3 weeks (n=30)	6 weeks (n=30)	8 weeks (n=30)
Left brain (normal)	1.039±0.112	1.056±0.094	1.004±0.034	1.021±0.057
Right brain (damage)	0.956±0.120	0.530±0.082[a,b]	0.316±0.012[a,b]	0.395±0.041[a,b]
Between groups	F=11.32, p=0.001			
Different time-points	F=7.12, p=0.025			
Between groups different time-points	F=9.53, p=0.014			

[a]p<0.05 vs. normal group; [b]p<0.05 vs. the expression of mRNA in left brain tissue.

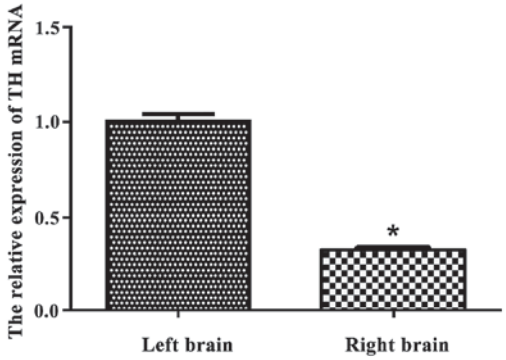

Figure 3. The relative expression of TH mRNA in brain tissues of PD model rats at 6 weeks after operation. *p<0.05.

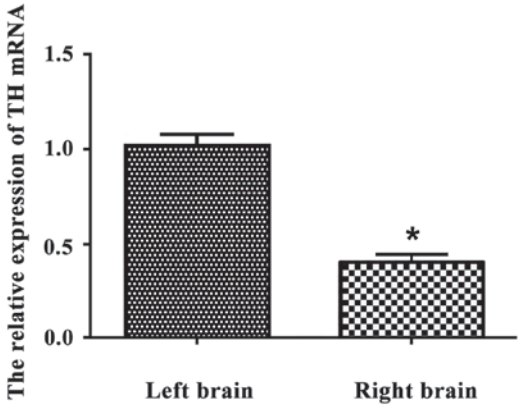

Figure 4. The relative expression of TH mRNA in brain tissues of PD model rats at 8 weeks after operation. *p<0.05.

for 6 weeks after operation (p<0.05). The relative expression amount was 1.004±0.034 in left brain tissue and 0.316±0.012 in right brain tissue.

The relative expression of TH mRNA in the left and right brain tissue of PD rats after operation for 8 weeks. As shown in Fig. 4, the relative expression of TH mRNA was 1.021±0.0578 in the left brain tissue and 0.395±0.041 in the right brain tissue in the PD rats. The expression of TH in left brain tissue was significantly more than that in the right brain tissue (p<0.05).

Comparison of relative expression of TH mRNA in both sides of brain tissue. The preoperative expression of TH mRNA had no significant difference in the sides of the brain tissue (p>0.05) by the analysis of variance. Comparatively, the expression of TH mRNA was significantly different between the sides of brain tissue after model establishment (F=11.64, p=0.001, F=7.48, p=0.021, F=9.59, p=0.016), with the expression decreased relatively. After 8 weeks of operation, the TH mRNA expression in damaged side of the brain tissue was somewhat increased, but it was still lower than the normal side (p<0.05) (Table II).

Discussion

PD is a common neurological degeneration in the elderly, with limb tremor and activity disorders as the main performance (8,9). PD is hidden, with slow progress, and has no obvious movement disorders at early stage. As the disease progress the patient may have symptoms such as static tremor, muscle rigidity, and slowness of movement (10,11). Although PD rarely endangers the lives of patients, it seriously reduces the quality of life and also increased the burden on patients on the family. So it is of great significance to improve the therapeutic effect of patients. The most important pathological changes in PD is the depletion of DA in striatum, and the degeneration of dopaminergic neurons in substantia nigra is an important reason for the reduction of striatum content. Studies suggested that oxidative stress, environmental factors, aging and genetic factors were all likely to be involved in the process of denatured death of PD dopaminergic neurons (12). TH, as a key pathway for the catalytic synthesis of catecholamines neurotransmitters, has an important role in regulating the rate of dopamine synthesis and is a rate-limiting enzyme for dopamine synthesis. The role of TH was played through the mutual coordination between the catalytic subregion and regulatory subregions, and then catecholamine neurotransmitters were synthesized. The study (13,14) showed that the expression of TH mRNA in substantia nigra of the brain of PD was decreased, with the relationship to its gene expression restriction. Therefore, if the expression change of TH was well understood in the PD patients, it could provide a theoretical basis for clinical treatment of PD.

In this study, fluorogenic quantitative PCR was used to determine the relative expression of TH mRNA in the brain of rats. It was found that the relative expression of TH mRNA in the left and right brain tissue had no significant difference in normal rats ($p>0.05$), with 1.039±0.112 and 0.956±0.120, respectively. With the extension of modeling time, the success rate of model establishment was significantly improved ($p<0.05$). The success rate of modeling and the relative expression of TH mRNA reached the peak value in the 6 weeks after operation. As the modeling time increased, the success rate of modeling and the relative expression of TH mRNA has not changed much compared to the rats at 6 weeks, with a P-value <0.05. That was to say, after 6 weeks model establishment, the model of PD rats was basically completed and met the standard of PD model in rats. The relative expression of TH mRNA in both sides of the brain tissue was significantly different ($p<0.05$) and decreased. The expression of TH mRNA in the injured brain tissue was significantly lower than that in normal rats. The results showed that the relative expression of TH mRNA in brain was of great significance in the pathogenesis of PD rats. With the impaired brain tissue in PD rats, the relative expression of TH mRNA also decreased. And the increase of the expression level after 8 weeks may be an experimental error, as the sample size is not big enough. Similarly, Zhao *et al* also found that the expression of TH in PD rats decreased compared to normal rats (15,16), which may be the important reason for dopaminergic neurons degeneration.

In conclusion, the decreased expression of TH mRNA in injured brain tissue was lower than that in normal brain tissue, which may be related to the occurrence and development of PD.

References

1. Yang QQ, Sun FL, Al HX, Zhang L and Wang W: Assessment of 6-hydroxydopamine-lesion induced behavioral alteration as a rat model of Parkinson's disease. Acta Neuropharm 1: 17-22, 2013.
2. Li J, Wang LN and Xiao HL: Effect of electroacupuncture on oxidative stress injury of dopaminergic neurons in rats with Parkinson's disease. Acupunct Res 39: 185-191, 2014.
3. Leem J: Acupuncture for motor symptom improvement in Parkinson's disease and the potential identification of respondersto acupuncture treatment. Integr Med Res 5: 332-335, 2006.
4. Shavali S, Combs CK and Ebadi M: Reactive macrophages increase oxidative stress and alpha-synuclein nitration during death of dopaminergic neuronal cells in co-culture: Relevance to Parkinson's disease. Neurochem Res 31: 85-94, 2006.
5. Feve AP: Current status of tyrosine hydroxylase in management of Parkinson's disease. CNS Neurol Disord Drug Targets 11: 450-455, 2012.
6. Meng XJ, Pang Q, Ding F, Xin T and Yang HA: Establishment of PD rat model by injecting Taclo sterically and directionally in to striatum. J ShandongUniv 52: 16-18, 2014 (In Chinese).
7. Kashkin VA, Shekunova EV, Makarova MN and Makarov VG: A study of combination treatment with nacom (levodopa + carbodope) and citicoline in the model of Parkinson disease inrats. Zh Nevrol Psikhiatr Im S S Korsakova 117: 59-63, 2017 (In Russian).
8. Roche M, Frison G, Brion JD, Provot O, Hamze A and Alami M: Csp^2-N bond formation via ligand-free Pd-catalyzed oxidative coupling reaction of *N*-tosylhydrazones and indole derivatives. J Org Chem 78: 8485-8495, 2013.
9. Anh Nguyen TN, Benatmane N, Fallahi V, Fang Y, Mohseni SM, Dumas RK and Åkerman J: $[Co/Pd]_4$-Co-Pd-NiFe spring magnets with highly tunable and uniform magnetization tilt angles. J Magn Magn Mater 324: 3929-3932, 2012.
10. McCabe K, Concannon RM, McKernan DP and Dowd E: Time-course of striatal Toll-like receptor expression inneurotoxic, environmental and inflammatory rat models of Parkinson's disease. J Neuroimmunol 310: 103-106, 2017.
11. Zheng LF, Zhang Y, Chen CL, Song J, Fan RF, Cai QQ, Wang ZY and Zhu JX: Alterations in TH- and ChAT-immunoreactive neurons in the DMV and gastric dysmotility in an LPS-induced PD rat model. Auton Neurosci 177: 194-198, 2013.
12. Michell AW, Tofaris GK, Gossage H, Tyers P, Spillantini MG and Barker RA: The effect of truncated human α-synuclein (1-120) on dopaminergic cells in a transgenic mouse model of Parkinson's Disease. Cell Transplant 16: 461-474, 2007.
13. Huang Y, Chang C, Zhang JW and Gao XQ: Alternation of proteins in brain of Parkinson's disease model rats after the transplantation of TH-NTN gene modified bone marrow mesenchymal stem cells. Zhonghua Yi Xue Za Zhi 92: 2353-2356, 2012 (In Chinese).
14. Ali F, Taresh S, Al-Nuzaily M, Mok PL, Ismail A and Ahmad S: Stem cells differentiation and probing their therapeutic applications in hematological disorders: A critical review. Eur Rev Med Pharmacol Sci 20: 4390-4400, 2016.
15. Thenral ST and Vanisree AJ: Peripheral assessment of the genes AQP4, PBP and TH in patients with Parkinson's disease. Neurochem Res 37: 512-515, 2012.
16. Zhao J: Effect of 3-n-butylphthalide on the expression of Bax caspase-8 and TH in the substantia nigra of PD rats. Chinese J Clin 15: 1288-1292, 2014 (In Chinese).