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Mitochondrial Dysfunction Appears in the Hippocampal Neurons of 3-Month-Old Alpha-Synuclein Transgenic Mice

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Abstract: Alpha-synuclein A53T transgenic mouse (A53T) is an essential tool to investigate the onsets and the extents of Parkinson's disease (PD) non-motor symptoms. Previous studies showed A53T mice exhibit a number of non-motor symptoms. However, the cause for these non-motor symptoms is still unknown. Our aim is to investigate the mitochondrial function in the hippocampal neurons of 3-month-old A53T mice. By enzyme-linked immunosorbent assay, we showed that the reactive oxidative species (ROS) level significantly increased in the hippocampus of A53T mice compared with that of the littermate controls. The cytochrome C content in the hippocampus of the A53T mice also increased. In addition, the ATP content decreased in the hippocampus of the A53T mice compared with the control. These results indicate that mitochondrial dysfunction appears in the hippocampal neurons of A53T mice, which may contribute to the cognitive disturbence in the young A53T mice.

Keywords: Parkinson's disease; A53T; Non-Motor Symptoms; Mitochondrial Dysfunction

Introduction:

Parkinson's disease (PD) is a progressive neurodegenerative disorder. The pathological hallmark of PD is the selective loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) and the formation of Lewy bodies (LBs), which are mainly comprised of alpha-synuclein (α-syn). Increasing evidence has indicated that PD patients suffer from both motor and non-motor symptoms (NMSs) (Ozdilek and Gunal, 2012). The NMSs, for instance, hyposmia, cognitive decline, depression and sleep disorders appears before or in parallel with motor deficits (Irvine et al., 2008; Ozansoy and Basak, 2013). Studies have shown that NMSs have even greater impact on patients than motor symptoms (Marinus and van Hilten, 2015; Mehndiratta et al., 2011). An in-depth study of NMSs of PD may contribute to the early diagnosis of the disease (Erro et al., 2012; Grinberg et al., 2010). However, to date, the pathogenesis of NMSs in PD remains unclear.

Alpha-synuclein A53T transgenic mouse is an animal model that studies the development of α-syn aggregation in Parkinson's disease. Due to the late appearance of motor symptoms, it becomes an ideal model for studying NMSs of PD (Kohl et al., 2012). In the present study, we used A53T mice and their littermate controls to determine the mitochrondrial function in the hippocampus and try to explore the possible mechanisms involved in the NMSs of PD.

Materials and Methods

Animals

Initially, the prp-a53t transgenic (tg) mice were obtained from the Jackson laboratory (J004479, USA) and were raised at the experimental animal center of Qingdao university. At 20 days, the offsprings were genotyped by semi-quantitative polymerase chain reaction (PCR) assay of DNA extracted from tails for told the difference from transgenic (tg) mice and wild-type (wt). Genotyping was performed as per Jackson laboratories protocols. The length of the PCR product was 248 bp. If expressions of the gene in a 248 bp, the mouse is tg, on the contrary is wt. All mice were housed with a temperature of 19 ± 2°C and an automatic 12/12 h light-dark cycled, as well as free access to food and water. All experiments were performed strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of Qingdao University.

Biochemical analysis of hippocampal homogenates

The mice were dicapitated and both sides of hippocampus were isolated. The samples were centrifuged at 1000g for 20 min at 4°C. ROS , cytochrome C and ATP levels were quantified by using ELISA kits. Assays were conducted according to the manufacturer's instructions in a 96-well microtiter plates that was incubated with polyclonal anti-mouse

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cytochrome C monoclonal antibody (JL13261, Jonln, CHN), ATP monoclonal antibody (JL20530, Jonln, CHN) or ROS monoclonal antibody (JL13481, Jonln, CHN). A series of 6 serially diluted standards (200, 100, 50, 25, 12.5, 0 nmol/L) and samples of 100 µL were dispensed with new disposable tips into appropriate wells. Then, dispensing 100 µL HRP-conjugated antibody into each well, and incubating for 1 h at 37°C. Briskly shake out the contents of the wells, and then adding 350 µL of Wash Buffer (20×) into each well. In addition, striking the wells sharply on absorbent paper to remove residual droplets and repeating five times. After washing, adding 50 µL substrate solution to each well. The 96-well plate was incubated at 37°C with darkness solution for 15 min. Stop solution of 50 µL was added into each well, thoroughly mix for 10 s. The absorbance (OD) of each well was detected at 450 ± 10 nm with a microtiter plate reader (Bio-Rad, Hercules, CA, USA). The mean absorbance (OD) value for each sample was used to determine the corresponding concentration from the standard curve. The concentration of the samples can be read directly from this standard curve.

Statistical analysis

The results expressed as mean ± S.E.M. The data were analyzed by unpaired t-test by using Prism Graph Pad 5.0 software (Graphpad Software, USA). $P<0.05$ was considered to be significant.

Results

The cytochrome C, ROS contents were elevated and ATP contents were reduced in the hippocampus of 3-month-old *tg* mice

By using ELISA, we first quatified the cytochrome C content in the hippocampus of the mice. As shown in figure 1A, the 3-month-old *tg* mice showed a significant elevation in cytochrome C content in the hippocumpus in comparison with the *wt* control. Then, we measured the ROS levels in the hippocampus of the mice, Statistical analysis indicated that the ROS contents were also elevated in the *tg* mice compared with the *wt* control (Fig. 1B). We further tested the ATP contents in the hippocampus of the mice. As shown in figure 1C, we showed a marked reduction in .ATP contents in the hippocampus of the *tg* mice.

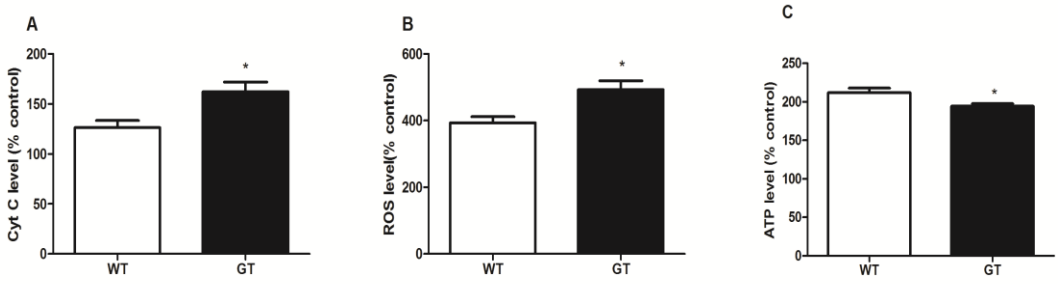

Figure 1. Cytochrome C, ROS, and ATP levels in the hippocampus of 3-month-old mice. Figure 1A and 1B showed that the contents of cytochrome C (figure A, q=0.0132, **P*<0.05, n=5) and ROS (figure B, q=0.0352, **P*<0.05, n=5) were significantly elevated in the hippocampus of *tg* mice, Figure 1C showed that the ATP level was significantly reduced in the hippocampus of *tg* mice compared with the wt control (figure C, q=0.0393, **P*<0.05, compared with wt mice, n=5).

Discussion

To date, non-motor symptoms, whether early diagnosis, prevention or treatment, are still relatively low compared to motor symptoms. Neither can they be fully understood by patients and clinicians, nor can they form more effective and reasonable treatment plans. And eventually become an important factor affecting the patient's quality of life in the near future, leading to the paralysis of patients in the long run(Charles et al., 2011; Hely et al., 2005). NMSs of PD involve multiple systems, such as the cardiovascular system, digestive system, autonomic nervous system, urinary system. The symptoms such as sleep disorders, emotional and cognitive dysfunction are relatively prominent (Arvanitakis et al., 2007). In addition, another study found that PD non-motor symptoms manifested as patient-specific, depending on the level of education and disease stage affected. And the same anti-Parkinson drugs do not completely suppress all non-motor symptoms and even exacerbate certain symptoms(Schaeffer and Berg, 2017). Although the NMSs of PD are getting more and more attention, the pathogenesis of many symptoms is not clear and has not been effectively controlled.

In this study, 3-month-old A53T mice were observed as a model of early Parkinson's disease. At this time, the mice did not show motor symptoms, however exhibited emotional and cognitive dysfunction. We have found that cytochrome C and ROS increased, and ATP levels decrease in the hippocampus of *tg* mice. These results indicate mitochondrial dysfunction in the hippocampus of A53T mice. Mitochondrial

dysfunction is associated with the development of NMSs in PD.

Mitochondria is an organelle that enveloped in two layers of membranes in a cell. The most important functions are energy metabolism and the energy required to maintain cell survival and exert biological effects. At the same time, mitochondria is also involved in the regulation of ROS, cell signal regulation, signaling cascades and other important biological processes. A variety of factors caused by mitochondrial dysfunction, affecting the normal regulation of the process, often have a serious impact on the cells. It has been reported that PD motor symptoms are associated with the striatum dopamine pathway, and that NMS is associated with other pathways (Jurado-Coronel et al., 2017), but mitochondrial dysfunction is implicated in both motor symptoms and non-motor symptoms. The classic PD model, often used the inhibitor of mitochondrial complexI, rotenone and parasol in laboratories which is intended to be consistent. Rats were injected with the drug showed tremor and postural instability and rotating with unilateral symptoms similar to Parkinson's patients, this evidence suggests that mitochondrial dysfunction leads to Parkinson's motor symptoms. And on the other hand there have evidence that NMS is associated with mitochondrial dysfunction, Bergamini C suggested that the supplementation of coenzyme Q could protect cells from energy loss (Bergamini et al., 2016), and the medium dose and large dose of oral coenzyme Q could reduce the updrs score and visual impairment, but showed no significant change in motor symptoms(Muller et al., 2003). Even research shows that gene PINK1/parkin is directly involved in regulating mitochondrial morphology and maintenance(Imai and Lu, 2011). Studies have shown that dysfunction of mitochondrial complex I in dopaminergic neurons promotes NMSs of PD and decreases dopamine levels(Choi et al., 2017). Other NMSs, such as sleep disturbances, are caused by oxidative stress and mitochondrial dysfunction (Willison et al., 2013).

However, the relationship between mitochondrial dysfunction and PD is complex and the mechanism of movement disorder and non-movement disorder is also interactive. In addition, during the clinical diagnosis and treatment of PD, the movement symptoms of PD patients can't be completely separated from the treatment of NMS, and even the two symptoms should be taken into account, which undoubtedly makes it more difficult for doctors and patients. In addition, in this trial, we still have some limitations, ROS and cytochrome C and other biochemical changes associated with mitochondrial function, not only to a certain extent is related to oxidative stress and inflammation. Therefore, it is of great significance to explore the mechanism of NMSs and alleviate the symptoms of NMS in patients with early diagnosis of Parkinson's disease and improve the quality of life of patients.

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CONFLICTS OF INTEREST

Declaration that the manuscript is original, has not been submitted to or is not under consideration by another publication and has not been previously published in any language or any form, including electronic. All of the authors declare that there have no conflicts of interests.

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