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Research article

Inhibition of HDAC6 increases acetylation of peroxiredoxin1/2 and ameliorates 6-OHDA induced dopaminergic injury

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ABSTRACT

Objective: Histone deacetylase 6 (HDAC6) has been regarded as an unusual HDAC because of its unique properties. It contains two deacetylase catalytic domains and one ubiquitin-binding domain, thus exerting both enzymatic and non-enzymatic actions on cellular function. To date, the ubiquitin-binding activity of HDAC6 has been implicated in several neurodegenerative disorders including Parkinson's disease (PD). However, the deacetylation effect of HDAC6 in PD has not been fully illustrated. Therefore, the aim of the present study was to explore the role of deacetyation activity of HDAC6 in PD.

Methods: We used an in vivo 6-OHDA induced PD model and a specific HDAC6 inhibitor tubastatin A to investigate the acetylation levels of peroxiredoxin1 (Prx1) and peroxiredoxin2 (Prx2) and to explore the effects of tubastain A on nigrostriatal dopaminergic system.

Results: Our results showed that expression of HDAC6 significantly increased in dopaminergic neurons after 6-OHDA injury. Acetylation levels of Prx1 and Prx2 decreased. Pharmacological inhibition of HDAC6 with specific inhibitor tubastatin A increased acetylation of Prx1 and Prx2, reduced ROS production and ameliorated dopaminergic neurotoxicity.

Conclusion: Our results for the first time provide evidence that HDAC6 medicated deacetylation of Prx1 and Prx2 contributes to oxidative injury in PD, suggesting that the development of specific HDAC6 inhibitor is required to develop more effective therapeutic strategies to treat PD.

1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, which is characterized by resting tremor, muscle rigidity, bradykinesia and postural instability. The most prominent pathological features are progressive loss of dopaminergic neurons in substantia nigra pars compacta (SNc) and loss of dopamine terminals in striatum (STR) [1,2]. Despite numerous studies demonstrating multiple pathogenic mechanisms such as inflammation, oxidative stress and excitotoxicity are involved in PD, there is currently no cure for this disease. Thus, research on the causes and treatments of this disease is crucial.

There is an increasing number of experimental studies have highlighted that histone deacetylases (HDACs) inhibitors exhibit neuroprotective properties in PD. Kontopoulos reported that toxicity of misfolded protein α-synuclein can be rescued by administration of HDAC inhibitors in both cell culture and transgenic flies [3]. HDAC inhibitor valproic acid is able to partially prevent striatal DA depletion and protect against substantia nigra dopaminergic cell loss [4]. HDAC inhibitors up-regulate GDNF and BDNF expression in astrocytes and protect dopaminergic neurons [5]. These results provide compelling evidence that HDACs may be relevant to pathogenesis in PD. HDACs are a family of enzymes that deacetylate lysine residues from histones as well as several other non-histone proteins. Currently, at least 18 mammal HDACs have been grouped into four classes [6]. Class I includes the constitutively expressed HDAC 1,2,3 and 8, mainly localized to the nucleus, and acts as transcriptional repressors by deacetylation of chromatin histone and other DNA-binding proteins. Class II is expressed stage and tissue specifically, subdivided into class IIa (HDAC4, 5, 7, and 9) and IIb (HDAC6 and 10) based on domain organization. Enzymes from class IIb are mostly found in the cytoplasm with a preference for non-histone proteins. HDAC11 is the sole member of class IV. These

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HDACs (class I, II and IV) share sequence similarity and are dependent on Zn^{2+} for enzymatic activity, usually referred as classical HDACs. Whereas class III, called sirtuins, which deacetylate non-histone proteins, are NAD^{+} dependent enzymes with different structural features [7]. However, it should be noted that currently available HDAC inhibitors are mostly non-selective and inhibit multiple HDAC proteins. Therefore, it is necessary to explore the role of individual HDACs in PD.

To date, few research activities have focused on the role of individual HDACs in PD and HDAC6 was suggested to be a promising target [8]. HDAC6 is regarded as an unusual HDAC because of its unique properties: predominantly located in the cytoplasm, containing two deacetylase catalytic domains and an ubiquitin-binding domain [9]. Investigations show that HDAC6 protects dopaminergic neurons against α-synuclein toxicity by promoting inclusion formation and facilitating autophagic degradation of these aggregated inclusions, this activity of HDAC6 relies on its ubiquitin-binding domain, which senses the accumulation of ubiquitinated misfolded proteins [10,11]. However, as we know that the unique feature of HDAC6 provides a support for its broad functions in mediating appropriate cell response. Its involvement in deacetylation gives HDAC6 an important role in the progression of neurodegenerative disorders [8]. Specific substrates of HDAC6 deacetylation are varied, such as peroxiredoxin1 (Prx1), peroxiredoxin2 (Prx2), tubulin, cortactin and HSP90 [12]. But the deacetylation effect of HDAC6 in PD has not been fully illustrated. Therefore, in this study, we used an in vivo 6-OHDA induced PD model and a specific HDAC6 inhibitor tubastatin A to investigate the deacetylation of Prx1 and Prx2 in PD and explored that pharmacological inhibition of HDAC6 with tubastain A can protect the nigrostriatal dopaminergic system.

2. Materials and methods

2.1. Animal studies

Animal models for PD were induced in eight-week-old male C57BL/6 mice. All procedures were pre-approved by Institutional Animal Care and Use Committee of Shandong University. Mice were randomly assigned to 3 experimental groups: sham group that was treated with vehicle only, 6-OHDA group, and 6-OHDA + tubastatin A group. For stereotaxic injections, the mice were anaesthetized with 40 mg/kg sodium pentobarbital and placed in a stereotaxic device. Then, 6 μg of 6-OHDA (162957, Sigma-Aldrich, dissolved in 2 μl of normal saline supplemented with 0.2% ascorbic acid) was injected into 2 different sites of right STR of the brain. The stereotaxic coordinates of the right STR were: bregma + 1.0 mm, lateral 2.1 mm, and ventral −2.9 mm, as well as bregma-0.3 mm, lateral 2.3 mm, and ventral −2.9 mm. Mice were sacrificed at different time points following 6-OHDA injection, and tissues were collected for biochemical or histological assessment. For tubastatin A treatment, mice were given 25 mg/kg tubastain A (S8049, Selleck) by intraperitoneal injection on seven consecutive days after 6-OHDA lesion.

2.2. Immunohistochemistry and immunofluorescence staining

Staining was performed as described [7]. For immunohistochemistry, tissue sections were incubated with TH antibody (MAB318, Millipore) and then incubated in horseradish peroxidase conjugated secondary antibody. The sections were visualized using diaminobenzidine. Digital images were collected in bright field microscope. For immunofluorescence staining, the sections were incubated with TH antibody (66334-1-Ig, ProteinTech) and HDAC6 antibody (12834-1-AP, ProteinTech), then incubated in fluorescent secondary antibody (Invitrogen) and digital images were collected in fluorescence microscope.

2.3. Western blot analysis

Protein extracts preparation and western blot analysis were performed as described previously [7]. The primary antibodies used in this study included TH (MAB318, Millipore), HDAC6 (07-732, Millipore), Prx1 (8499, Cell signaling), Prx2 (ab109367, Abcam), and acetylated lysine (9441, Cell signaling). To document the loading controls, the membranes were probed with a primary antibody against housekeeping protein GAPDH (TA-08, ZSGB-Bio). Densitometry analyses were performed using AlphaEaseFC software.

2.4. Real-time RT-PCR

Total RNA was prepared from tissues using TRIzol reagent and mRNA levels were analyzed by real-time RT-PCR using a Bio-Rad iCycler system [7]. The specific primers for target genes were as follows: HDAC6 forward: TCTTTCTGGTGCTTGTCTC and reverse: AGTGTGAGCCAGGATGTAG; GAPDH forward: TACCCGGACTGGATTCTACG and reverse: AAGTTGG TGGGCTGTCAATC. The cycle threshold (Ct) values were used for calculation of gene expression in accordance with the △△Ct method.

2.5. Immunoprecipitation

Brain extracts were homogenized in NP-40 buffer with added protease inhibitors, followed by centrifugation and determination of the protein concentration. Immunoprecipitaiton was performed by incubating sample protein with a kit containing Dynabeads Protein G (NOVEX IP Kit), according to manufacturer's protocol. Briefly, the primary antibody Prx1 (15816-1-AP, ProteinTech) or Prx2 (10545-2-AP, ProteinTech) was incubated with Dynabeads for 30 min at room temperature, followed by addition of sample containing the antigen and further incubation at 4 °C overnight to allow antigen to bind to the Dynabeads-antibody complex. The beads were collected by the magnet and washed 3 times, then boiled with 5 × reducing SDS sample buffer in Elution buffer for 10 min, separated on 15% SDS–PAGE, and analyzed by western blotting using the acetylated lysine antibody or Prx1 and Prx2 antibody described above.

2.6. ROS determination

Dihydroethidium (DHE) staining was used to assess reactive oxygen species (ROS) production. Briefly, cryosections were incubated with 5 μM DHE (Beyotime) for 30 min at 37 °C in a light-protected humidified container and images were captured with fluorescence microscope. The intensity of red fluorescence, representing superoxide production, was measured using the Image-Pro Plus 6.0 software. Four sections from each group were analyzed using this procedure and the average superoxide induced DHE fluorescence was calculated.

2.7. Behavioral testing

Behavior testing was measured according to a method described previously [13]. Apomorphine-induced rotations were monitored post 6-OHDA lesioning. Mice given a subcutaneous injection of apomorphine were placed individually in a plastic container in a quiet isolated room. Quantitative analyses of completed (360°) left and right rotations were made by an investigator blinded to the experimental conditions.

2.8. Statistics

Data are expressed as means ± SEM. The significance of the differences in mean values between and within multiple groups was examined by one-way ANOVA followed by Bonferroni post test. $p < 0.05$ was considered statistically significant.