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Research Paper

2,4 DNP improves motor function, preserves medium spiny neuronal identity, and reduces oxidative stress in a mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the first exon of the gene huntingtin. There is no treatment to prevent or delay the disease course of HD currently. Oxidative stress and mitochondrial dysfunction have emerged as key determinants of the disease progression in HD. Therefore, counteracting mutant huntingtin (mHtt)-induced oxidative stress and mitochondrial dysfunction appears as a new approach to treat this devastating disease. Interestingly, mild mitochondrial uncoupling improves neuronal resistance to stress and facilitates neuronal survival. Mild mitochondrial uncoupling can be induced by the proper dose of 2,4-dinitrophenol (DNP), a proton ionophore that was previously used for weight loss. In this study, we evaluated the effects of chronic administration of DNP at three doses (0.5, 1, 5 mg/kg/day) on mHtt-induced behavioral deficits and cellular abnormalities in the N171-82Q HD mouse model. DNP at a low dose (1 mg/kg/day) significantly improved motor function and preserved medium spiny neuronal marker DARPP32 and postsynaptic protein PSD95 in the striatum of HD mice. Further mechanistic study suggests that DNP at this dose reduced oxidative stress in HD mice, which was indicated by reduced levels of F2-isoprostanes in the brain of HD mice treated with DNP. Our data indicated that DNP provided behavioral benefit and neuroprotective effect at a weight neutral dose in HD mice, suggesting that the potential value of repositioning DNP to HD treatment is warranted in well-controlled clinical trials in HD.

1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder, caused by an abnormal expansion of a CAG repeat in exon 1 of the gene encoding the huntingtin protein (Htt) (The Huntington's disease Collaborative Research group, 1993). Individuals who have 36 CAG repeats or more in the *huntingtin* gene develop the clinical symptoms, including motor, cognition, and mental abnormalities that cause a progressive loss of functional capacity and shortened life span (Ross and Tabrizi, 2011). The mutation results in a polyglutamine tract at the N-terminal of the mutant Htt protein. The pathology of the disease has been attributed to toxic gain of functions for the mutant Htt as well as loss of beneficial functions of wild type Htt protein (Ross and Tabrizi, 2011). The major neuropathology in HD is the selective loss of medium spiny neurons in the caudate putamen of patients (Graveland et al., 1985; Reiner et al., 1988; Vonsattel et al., 1985). Despite remarkable progress in our understanding of this disease, the molecular logic connecting mutant Htt-mediated neuronal dysfunction and pathological symptoms remains unclear.

Accumulating data has indicated that oxidative stress plays a crucial role in HD pathogenesis (Kumar and Ratan, 2016). The two major factors that make the brain more prone to oxidative damage are high lipid concentrations and high energy requirements (Walker, 2007).

Abbreviations: DNP, 2,4-dinitrophenol; HD, Huntington's disease; mHtt, mutant huntingtin protein; ROS, reactive oxygen species; UCP, uncoupling protein.

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Mutant Htt can also serve as the source of reactive oxygen species (ROS) (Rotblat et al., 2014). Moreover, the extensive oxidative DNA damage has also been reported in HD models (Browne, 2008; Gil-Mohapel et al., 2014; Goula et al., 2009). DNA oxidation was detected in serum, blood and leukocytes from HD patients (Chen et al., 2007; Hersch et al., 2006; Tunez et al., 2011). Impairment in the electron transport chain and mitochondrial dysfunction are the major mechanisms involved in the ROS mediated pathogenesis of HD (Sayre et al., 2008; Trushina and McMurray, 2007). HD patients showed an increased level of oxidative stress markers accompanied by a decrease in antioxidant status compared to healthy subjects (Chen et al., 2007; Montine et al., 1999). Both in vivo and in vitro studies have documented the protective role of various natural antioxidant products or synthetic entities in the prevention of HD (Johri and Beal, 2012; Stack et al., 2008). However, the neuroprotective treatment for HD patients has not yet been found.

Dietary restriction (DR) without malnutrition is a well-tested intervention in aging and models of neurodegenerative disorders (Arslan-Ergul et al., 2013; Hunt et al., 2006; Lopez-Lluch and Navas, 2016; Mattson, 2008; Sohal and Forster, 2014). We have demonstrated that HD mice maintained on a DR regimen exhibited significantly improved motor function and lessened brain pathology (Duan et al., 2003). Mild uncoupling increases metabolic inefficiency, effectively "restricting" caloric conversion into biological work; this is a mechanism for DR-mediated beneficial effects. Therefore, chemical mitochondrial uncoupler(s) may represent effective DR mimetic. The protonophore 2,4-dinitrophenol (DNP), which was extensively used as an obesity treatment in the 1930s (Colman, 2007), has been increasingly utilized as a putative DR mimetic. Later it was learned that DNP is a mitochondrial uncoupler (Geisler, 2011; Simkins, 1937). Promisingly, in flies and mice, DNP has been shown to increase lifespan, accompanied by decreases in oxidative damage (Caldeira da Silva et al., 2008; Padalko, 2005). In addition, chronic administration of DNP significantly improved learning and memory in mice (Geisler et al., 2016) and is beneficial in a stroke model by lowering ROS (Korde et al., 2005). In this study, we evaluated the effects of DNP on mutant Htt-associated abnormalities in a well-characterized N171-82Q HD mouse model. Our results indicate that chronic administration of appropriate low dose DNP improved motor function, preserved medium spiny neuronal identity, and reduced oxidative stress in HD mice, suggesting that mild mitochondrial uncoupling might be a new therapeutic strategy for HD.

2. Materials and methods

2.1. Mice

Male N171-82Q HD mice were mated to female B6C3F1/J mice (Jackson Laboratory, ME) to generate the experimental mice. Male mice were used in our current study, because we found gender-dependent phenotypic differences in N171-82Q HD mice, therefore we use male N171-82Q mice for this preclinical study as we did previously (Jiang et al., 2013; Jiang et al., 2012). Mice were randomly divided into six groups. DNP or placebo (1% sodium bicarbonate, pH 7.0) was orally given to the mice by gavage from 8 weeks old to the end of the study. Animals were housed under specific pathogen-free conditions with a reversed 12-h light/dark cycle maintained at 23 °C and provided with food and water ad libitum. All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Johns Hopkins University Animal Care and Use Committee.

2.2. Drug preparation

2,4-DNP is slightly acidic and was dissolved by adding sodium bicarbonate. Briefly, DNP was added to water first and 90 mg/mL sodium bicarbonate solution was added at 1% to each dosing solution. Final drug concentrations were 0.5 mg/mL (for 5 mg/kg dose), 0.1 mg/mL (for 1 mg/kg dose) and 0.05 mg/mL (for 0.5 mg/kg dose). The DNP solution was stored at room temperature and kept from light. Fresh DNP solutions were made every three days.

2.3. Behavioral tests

Motor function was assessed on an 80-cm long and 5-mm wide square-shaped balance beam. The balance beam was mounted on 50-cm high supports. A bright light illuminated the start platform, and a darkened enclosed 1728 cm^3 escape box (12 × 12 × 12 cm) was situated at the end of the beam. Disposable pads placed under the beam provided cushioning if a mouse fell off the beam. Mice were trained to walk across the beam twice at least 1 h prior to testing. If a mouse stopped during training, the tail was gently pressed to encourage movement. After the training trial, mice were left undisturbed for at least an hour before testing. The time for each mouse to traverse the balance beam was recorded with a 60-s maximum cut-off, and falls were scored as 60 s. In addition to the 5 mm balance beam, the tapered beam was also used to evaluate hindlimb function. To increase sensitivity of the task and to encourage the mice to run the beam reliably, the beam was angled at 17° from the horizontal such that the mouse was able to run uphill. Our apparatus also has a goal box, as the use of a goal box can be beneficial for expediting training and increasing the reliability of the test performance. As the beam is elevated above ground level, a soft landing area is essential beneath the beam, which in our laboratory consists of numerous bench pads. The traverse time when the mouse crossed the start line and ended was recorded.

2.4. Survival

Survival was monitored daily by experienced investigators (B.W and Q.P). The mice were considered at the end of life when they were unable to right themselves after being placed on their backs and initiate movement after being gently prodded for 30 s.

2.5. In vivo structural MRI acquisition and quantification of brain volume

In vivo structural MRI scans were performed on a horizontal 9.4T magnetic resonance imager (Bruker Biospin, Billerica) with a triple-axis gradient and an animal imaging probe. The detailed image capture and analysis were described in our previous study (Cheng et al., 2011). Briefly, mice were anesthetized with 1% isoflurane, respiration was monitored and the temperature was maintained during the entire scan. Images were acquired by a three-dimensional T2-weighted fast spin echo sequence with the following parameters: echo time (TE)/repetition time (TR) = 40/700 ms, resolution = 0.1 mm × 0.1 mm × 0.1 mm, echo train length = 4, number of average = 2 and flip angle = 40°. The imaging resolution and contrast were sufficient for automatic volumetric characterization of the mouse brains and substructures. The intensity-normalized images were submitted by the Diffeomap software to a linux cluster, which runs Large Deformation Diffeomorphic Metric Mapping (LDDMM). The transformations encode morphological differences between subject and template images and can be analyzed with deformation-based morphometry to detect regional changes in brain volume.

2.6. Protein extraction and Western blot analysis

Mouse brain tissues were collected at 4-h following the last DNP administration. Striatum was homogenized in RIPA buffer (Sigma) containing 50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and freshly prepared protease inhibitor (1:100, Sigma) and/or phosphatase inhibitor (1:100, Sigma). Samples were then centrifuged at 14,000 × *g* for 15 min at 4 °C and supernatant fractions were collected. Protein concentration was determined by the Micro BCA protein assay kit (Pierce