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Sertraline slows disease progression and increases neurogenesis in N171-82Q mouse model of Huntington's disease

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Huntington's disease (HD) is an inherited progressive neurodegenerative disorder resulting from CAG repeat expansion in the gene that encodes for the protein huntingtin. To identify neuroprotective compound (s) that can slow down disease progression and can be administered long term with few side effects in Huntington's disease, we investigated the effect of sertraline, a selective serotonin reuptake inhibitor (SSRI) which has been shown to upregulate BDNF levels in rodent brains. We report here that in HD mice sertraline increased BDNF levels, preserved chaperone protein HSP70 and Bcl-2 levels in brains, attenuated the progression of brain atrophy and behavioral abnormalities and thereby increased survival. Sertraline also enhanced neurogenesis, which appeared to be responsible for mediating the beneficial effects of sertraline in HD mice. Additionally, the effective levels of sertraline are comparable to the safe levels achievable in humans. The findings suggest that sertraline is a potential candidate for treatment of HD patients.

Keywords: SSRI; Serotonin; BDNF; Huntington's disease; Neurogenesis

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder in which neurons in the striatum and the cerebral cortex degenerate, resulting in abnormal involuntary movements (chorea), and psychiatric and cognitive abnormalities (Reiner et al., 1998; Vonsattel et al., 1985; Myers et al., 1988). Death usually occurs within 10–20 years after onset of the first clinical symptoms. Although the mechanism of neuronal degeneration in HD is unclear, it is thought that disease onset and progression may be involved in transcription dysregulation (Cha, 2003; Sugars and Rubinsztein, 2003) and deficits of neurotrophic factors (Zuccato et al., 2001, 2005; Zuccato and Cattaneo, 2007; Ferrigno and Silver, 2000; Gauthier et al., 2004; Cowan and Raymond, 2006), with cumulative neuronal loss.

It has been demonstrated that levels of brain-derived neurotrophic factor (BDNF) are decreased in brain cells of HD patients and mutant huntingtin transgenic mice. Although the crucial mechanism(s) of neuronal death induced by mutant htt is still unclear, mutant huntingtin promotes neuronal degeneration, in part by suppressing BDNF transcription and/or blocking axonal transport of BDNF from cerebral cortex to striatum (Zuccato et al., 2001, 2003; Gauthier et al., 2004). Other studies have shown that BDNF can protect neurons against insults relevant to the pathogenesis of HD (Bemelmans et al., 1999; Kells et al., 2004; Canals et al., 2004; Cepeda et al., 2004; Pineda et al., 2005; Zuccato et al., 2005; Lynch et al., 2007). In addition to promoting neuronal survival, BDNF also regulates neurogenesis (Duman, 2004; Cotman and Berchtold 2002). Altered neurogenesis occurred in mouse models of HD and in postmortem brains in humans with the disease (Phillips et al., 2006, 2005; Grote et al., 2005; Gil et al., 2005; Tattersfield et al., 2004; Lazic et al., 2004; Curtis et al., 2003). Mutant huntingtin also interrupts other cell-protective systems, such as chaperone protein, leading to dysfunction of the protein refolding system (Landles and Bates 2004).

There is currently no therapy to delay onset or prevent disease progression in HD patients. Promising approaches have been reported in preclinical trials, such as treating with FGF-2 (Jin et al., 2005) and GDNF (McBride et al., 2006), which prevented neuronal death and dysfunction, or by replacing lost neurons by transplanting embryonic stem cells (Conti et al 2006). Nonetheless, most of these approaches are associated with ethical, technical, and immunological problems for application to human patients (Rosser and Dunnett, 2003). Searching for drugs that can delay onset and/or slow disease

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progression with few side effects after administration for long periods will be valuable for treating HD patients.

Selective serotonin reuptake inhibitors (SSRIs), a class of drugs that is widely used for the treatment of patients with depression and severe anxiety disorders, has been shown to increase BDNF expression in brain (Nibuya et al., 1995, 1996; Moltzen and Bang-Andersen, 2006). SSRIs can also stimulate neurogenesis and protect neurons against metabolic/oxidative insults and processes known to be responsive to BDNF (Malberg and Blendy, 2005). Moreover, SSRIs are very safe and well tolerated over long periods of administration. We previously reported that an SSRI, paroxetine, is beneficial in HD mice (Duan et al., 2004). In the present study we investigated the effect of another widely prescribed SSRI, sertraline, on disease progression and molecular mechanisms in N171-82Q HD mice. Sertraline has been reported to be effective as treatment for depression (Slaughter et al., 2001), obsessive compulsive disorders (Patzold and Brune, 2002), and severe aggressiveness (Ranen et al., 1996) in HD patients. In addition to its anti-psychiatric effect, however, whether sertraline is neuroprotective and whether it prevents neurodegeneration and disease progression of HD, to our knowledge, have not been studied.

We show here that sertraline increases BDNF levels, preserves chaperone protein HSP70 levels and anti-apoptotic protein Bcl-2 levels, restores depleted serotonin levels, retards motor behavioral impairment, enhances neurogenesis and increases survival in HD mice. The increased neurogenesis apparently mediates its beneficial effects in HD. The effective levels of sertraline in the blood are comparable to the levels that are safe and achievable in humans. Taken together, we provide evidence that sertraline is neuroprotective in HD and the rationale for further clinical trials of SSRIs in HD patients.

Materials and methods

Mice and drug administration

All animal experiments were performed according to procedures approved by the Institutional Animal Care and Use Committee. N171-82Q transgenic HD mice were mated to hybrid mice (C3H/HEJ × C57BL/6J F1, Taconic, NY). All HD mice were housed in cage conditions including an orange mouse igloo and a green nylabone setting in the cage, and wet mash was provided to all mice starting at weaning. We used male HD mice for all our studies since we found that there is a significant variability in all phenotypes between male and female HD mice (Duan et al., 2003). Each group contained 15 mice at the beginning of experiments for survival and behavioral tests. Sertraline was freshly prepared by dissolving in vehicle 0.2% Tween-80 and administered to 12-week-old mice by daily intraperitoneal injection until the end of studies.

Behavioral test

All mice were randomly divided into each group. Nontransgenic control mice are wild type littermates of HD mice. Each group contained 15 mice at the beginning of the rotarod test. We used the same set of animals for survival analyses and motor performance tests.

Motor behavioral performance was assessed using a rotarod apparatus with an accelerating speed (Columbus Instrument, OH). Each mouse was trained at speed of 4 rpm for 5 min, the training session was followed by a 1-hour rest period in the home cage. Mice were then placed back on the rotarod apparatus for three trials (maximal 3 min at accelerating speed 4–40 rpm) separated by a 30-minute rest period between trials. Mice were tested for 3 consecutive days, by which time a steady baseline level of performance was attained.

X-ray irradiation to eliminate neurogenesis

Radiation was delivered using the in-house small animal radiation research platform (SARRP) (Wong et al., submitted for publication). The SARRP consists of an industrial X-ray tube with an adjustable anode voltage up to 225 kVp which delivers a beam through a collimator system. The animals were held on a robotic platform which permitted motion along the three principal axes as well as rotation. For these experiments, mice were positioned prone in a custom-designed stereotactic head holder system which was suitable for use of isoflurane anesthesia (2%). The beam was collimated using a custom lead cutout 8 × 10 mm at the isocenter (35 cm from the X-ray source). Using the robotic stages, the mice were positioned under the collimator. This set-up was intended to irradiate both the hippocampus and subventricular zone (SVZ) (see Fig. 6a), regions that are known to harbor neuroprogenitor cells (Santarelli et al., 2003) and are known to be sensitive to radiation (Monje et al., 2002), while sparing the olfactory bulb and other brain parenchyma. Positioning was verified using radiographs taken at a source voltage of 100 kVp. After alignment, a single radiation dose of 10 Gy was delivered in 6.5 min. This radiation dose was measured using gafchromic EBT film and a water-equivalent solid water phantom arranged in a set-up to match the experimental conditions (for further details on film dosimetry see Deng et al., 2007). Sham radiations were performed by the same anesthesia procedure as above without X-ray delivery.

BrdU administration, immunohistochemistry, and stereological quantification

For the neurogenesis study, all mice received twice daily injections of 50 mg/kg BrdU (5-bromo-2-deoxyuridine; Sigma) in sterile 0.9% NaCl solution at 8 h apart for 5 consecutive days. Animals were perfused at 3 days after the last BrdU injection for assessing proliferation, and perfused at 3 weeks after the last injection for study of survival of newly generated cells. BrdU immunostaining was performed as we previously described (Lee et al., 2002). Briefly, coronal brain sections (40 μm) were made, endogenous peroxidases were quenched with 0.6% H_2O_2 for 30 min, the sections were incubated in 2 N HCl for 30 min at 37 °C and washed in 0.1 M borate buffer (pH 8.5) for 10 min. For light microscope quantification of BrdU-labeled cells, a series of every sixth 40-μm section was used. Sections were blocked in 1 × TBS containing 3% horse serum and 0.3% Triton X-100 for 1 h, followed by incubation in anti-BrdU antibody (1:200, BD Biosciences) or EM48 (1:200, rabbit polyclonal anti-polyglutamine antibody, Millipore) overnight at 4 °C. Sections were then incubated with biotinylated anti-mouse IgG for 1 h at room temperature. The immunoreactive product was detected using the vectastain ABC kit enhancing system (Vector Laboratory. CA) with diaminobenzadine (DAB) as substrate. Sections were counterstained with Nissl substrate for stereological quantification.

Stereology counting was performed in serial coronal sections on blind-coded slides. The numbers of BrdU-positive cells in the subgranule zone (SGZ) of the dentate gyrus (DG) were assessed by counting all positive cells in sections at the levels of the bregma