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Research report

Beneficial effects of glatiramer acetate in Huntington's disease mouse models: Evidence for BDNF-elevating and immunomodulatory mechanisms

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ABSTRACT

Huntington's disease (HD) is a fatal, neurodegenerative movement disorder that has no cure and few treatment options. In these preclinical studies, we tested the effects of chronic treatment of glatiramer acetate (GA; Copaxone®), an FDA-approved drug used as first-line therapy for MS, in two different HD mouse models, and explored potential mechanisms of action of drug efficacy. Groups of CAG140 knock-in and N171-82Q transgenic mice were treated with GA for up to 1 year of age (CAG140 knock-in mice) or 20 weeks (N171-82Q mice). Various behavioral assays were measured over the course of drug treatment whereby GA treatment delayed the onset and reduced the severity of HD behavioral symptoms in both mouse models. The beneficial actions of GA were associated with elevated levels of promoter I- and IV-driven brain-derived neurotrophic factor (*Bdnf*) expression and reduced levels of cytokines, in particular, interleukins IL4 and IL12, in the brains of HD mice. In addition, the GA-induced effects on BDNF, IL4 and IL12 levels were detected in plasma from drug-treated mice and rats, suggesting utility as a peripheral biomarker of treatment effectiveness. These preclinical studies support the use of GA as a relevant clinical therapy for HD patients.

1. Introduction

Huntington's disease (HD) is caused by a CAG repeat expansion mutation in the Huntingtin (*HTT) gene*, which leads to progressive movement dysfunction, cognitive impairment and behavioral abnormalities (Group, 1993). Clinical signs of HD typically emerge in adult ages (30–40 s), but juvenile-onset and late-onset cases are also observed, with death following approximately 15 years after disease onset. Since the identification of the *HTT* gene in 1993, HD has been the focus of extensive preclinical and clinical research in efforts to find a treatment for this devastating disorder, and although there are many promising candidates on the horizon, few are clinically available.

Glatiramer acetate (GA; copolymer 1; Copaxone®) is an FDA-approved drug used as first-line treatment for relapsing-remitting multiple sclerosis (MS). It is a random polymer composed of four amino acids that are found in myelin basic protein, namely glutamic acid, lysine, alanine, and tyrosine. The mechanisms of action of GA in MS are not fully understood, but are thought to involve immunomodulatory effects, via a downregulation of proinflammatory cytokines (Neuhaus et al., 2001; Blanco et al., 2006) and/or neuroprotective effects, via increased release of brain-derived neurotrophic factor (BDNF) from immune cells (Aharoni et al., 2005; Chen et al., 2003; Ziemssen et al., 2002). Neuroprotective and immunomodulatory mechanisms have relevance not only for the treatment of MS, but have also been implicated as target pathways for HD. For example, reduced BDNF expression is thought to play a crucial role in HD pathogenesis, whereby decreased expression and transport of BDNF has been observed in brain tissue from human HD patients (Ferrer et al., 2000; Zuccato et al., 2001) and in several different mouse models transgenic for mutant huntingtin, including R6/2, YAC72 and N171-82Q transgenic mice (Duan et al., 2003; Zhang et al., 2003; Zuccato et al., 2001). Accordingly, restoring striatal BDNF levels has been shown to have therapeutic effects in HD mouse models (Gharami et al., 2008; Giampa et al., 2013; Xie et al., 2010).

Additionally, data are emerging to implicate inflammation and immune dysfunction as playing an important role in the patho-

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genic mechanisms of cell death in HD (Ellrichmann et al., 2013; Silvestroni et al., 2009). Although inflammation is not an initiating factor in the pathology of HD, growing evidence indicates that inflammatory responses involving astrocytes, microglia, as well as the peripheral immune system contributes to disease progression (Dalrymple et al., 2007; Bjorkqvist et al., 2008; Silvestroni et al., 2009). For example, specific increases in proinflammatory cytokines, including interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor alpha (TNFα), have been found in postmortem striatum from HD patients and mouse models (Dalrymple et al., 2007; Bjorkqvist et al., 2008; Silvestroni et al., 2009), as well as in plasma from human HD patients (Dalrymple et al., 2007; Chang et al., 2015). Given these overlapping disease mechanisms between MS and HD, we have investigated whether GA might be a relevant treatment option for HD.

In our early studies, we demonstrated that GA can increase BDNF levels in cultured striatal cells and in striatal tissue after short-term (5-day) *in vivo* administration (Corey-Bloom et al., 2014). In the current study, we further carried out preclinical studies to assess the efficacy of chronic GA administration in two different HD mouse models, CAG140 knock-in (KI) mice and N171-82Q transgenic mice, and to explore potential mechanisms of action for GA's beneficial effects. Our results demonstrate that GA treatment improved disease phenotypes in both HD mouse models. The beneficial effects of GA were associated with modifying levels of BDNF and interleukins, not only in brain tissue, but also in plasma. These findings strongly suggest that GA might represent a relevant clinical therapy for HD patients and the blood measurements of BDNF and IL4/IL12 might serve as markers for drug effectiveness.

2. Materials and methods

2.1. Animals and drug treatments

All animals were housed n = 3–4 per cage, and maintained on a reverse 12-h light/dark cycle with lights on at 9:00 p.m. and free access to food (normal rodent chow) and water. CAG140 KI mice contain a chimeric mouse/human exon 1 with 140 CAG repeats inserted into the mouse gene by homologous targeting (Menalled et al., 2003), and were maintained by breeding of heterozygote pairs. CAG140 KI mice were genotyped at 4 weeks of age to determine homozygosity for the CAG140 mutation. The CAG repeat lengths in these mice has been verified by commercial genotyping (Laragen Inc, Culver City, CA) and found to be 130 ± 3 CAGs (reduced from the original description of 140 CAGs) (Menalled et al., 2003). Previous studies on these mice have reported climbing deficits in these mice as early as 6 weeks of age and rotarod deficits by 4 months of age (Hickey et al., 2008, 2012). Transgenic N171-82Q HD mice were maintained by breeding heterozygous N171-82Q males with C3B6F1 females (Jackson Laboratories). At the age of 4 weeks, mice were genotyped according to the Jackson Laboratories protocols. The CAG repeat length in these mice is 82 ± 1 CAGs (Laragen, Los Angeles, CA). The lifespan of the N171-82Q mice is ~20 weeks in our colony, with HD-like symptoms beginning at ~8 weeks of age. Litters of these mice were assigned randomly to the various experimental groups to achieve a minimum of n = 8 per group for behavioral testing. To avoid litter effects, mice from the same litter were evenly split into vehicle- and drug-treated groups. Power calculations show that groups of 8 mice are sufficient to have a 90% chance of detecting a 25% improvement in rotarod behavior, which is a minimal expected level of improvement [n = log(0.1)/log(0.75) = 8]. All other tests are expected to reveal >25% improvement as a result of drug treatment.

Groups of CAG140 KI mice (n = 17–20 per genotype and drug condition; 50:50, M:F) were injected s.c. with GA (0.625 mg/-mouse) or an equal amount of vehicle (40% mannitol) once a day, 3× per week, beginning at 3 months of age. One half of the mice were sacrificed at 7 months of age, with the remaining mice (n = 8–10 per group) sacrificed at 1 year of age. Mice were sacrificed using isoflurane overdose in a bell jar. For the N171-82Q transgenic line, groups of mice (n = 8–10 per genotype and drug treatment; 50:50, M:F) were injected with 1 mg/mouse GA, 5×/week, beginning at 8 weeks of age until 20 weeks of age. Body weight was recorded at each injection. The doses used for injection were based on previous studies in mice where ranges of 0.15 to 2 mg/mouse have been used (Moore et al., 2014; Poittevin et al., 2013; Smirnov et al., 2013; Teitelbaum et al., 2004). Further, extrapolation of the dose from animals to humans requires consideration of body surface area, which is related to metabolic rate of an animal (Nair and Jacob, 2016). Considering body surface, our mouse dose correlates to 75 mg/m^2, which is ~3-fold higher than a typical human dose of 23.8 mg/m^2 (Nair and Jacob, 2016). Mice were sacrificed 4 hours after the final injection, brains rapidly removed and trunk blood collected into heparin-coated tubes. For rat studies, Sprague-Dawley rats (Charles River) were used. At the age of 6 weeks, four groups of rats (n = 4 per group) received daily injections of GA (i.p.) for 5 days. Rats were sacrificed 4 h after the final injection by isoflurane overdose, brains rapidly removed and trunk blood collected into heparin-coated tubes. All procedures were in strict accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals.*

2.2. Behavioral tests

All mice were tested in the following behavioral paradigms at the time points indicated: *Open field activity (OFA) test:* OFA was measured in a square plexiglass chamber (27.3 cm × 27.3 cm) (Med Associates INC). The test chamber is divided into 16 squares (12 outer and 4 inner) of equal areas and includes three 16 photobeam I/R arrays to automatically record movement. Eight behavioral parameters (ambulatory counts, ambulatory time, stereotypic counts, stereotypic time, vertical counts, vertical time, resting time and jumping time) were automatically recorded during a 10 min observation period. Mice were tested in the open field at 18 weeks of age (N171-82Q transgenic mice) or 6 and 8.5 months (CAG140 KI mice). *Rotarod test:* Animals were tested on an AccuRotor rotarod (AccuScan Instruments) during the dark phase of the 12 h light-dark cycle using an accelerating rotation paradigm (4–40 rpm over 10 min). The time of fall was recorded. During the course of the experiment, mice were tested in a set of four trials each day for four consecutive days. Mice were tested at 15 weeks (N171-82Q transgenic mice) or 6, 9 and 12 months (CAG140 KI mice). *Climbing test:* To assess climbing activity, mice were placed on the floor of a wire cylinder (8″ height × 4″ diameter) for 5 min. Climbing was recorded when two or four paws of the mouse are off the floor of the testing bench. Mice were tested in climbing at 11 and 17 weeks (N171-82Q transgenic mice) or 5.5 and 7 months (CAG140 KI mice). *Alternating T-maze*. The alternating T-maze test was performed as described previously (Jia et al., 2015) at 8 months of age. The T-maze is made of transparent Plexiglas with a central arm (75 cm long × 12 cm wide × 20 cm high) and two lateral arms (32 cm long × 12 cm wide × 20 cm high) positioned at a 90° angle relative to the central arm. Forced alternation was used for the T-maze training. For T-maze testing, mice were provided an initial free choice for either arm of the maze, and the percentage of alternation over the next nine trials was determined. *Grip strength.* Grip strength was determined at 1 year of age using a Grip Strength Meter consisting of a baseplate, a