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Research report

Intravenous immunoglobulin ameliorates motor and cognitive deficits and neuropathology in R6/2 mouse model of Huntington's disease by decreasing mutant huntingtin protein level and normalizing NF-κB signaling pathway

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ABSTRACT

Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by progressive movement disorders and cognitive deficits, which is caused by a CAG-repeat expansion encoding an extended polyglutamine (polyQ) tract in the huntingtin protein (HTT). Reduction of mutant HTT levels and inhibition of neuroinflammation has been proposed as a major therapeutic strategy in treating HD. Intravenous immunoglobulin (IVIg) therapy has been firmly established for the treatment of several autoimmune or inflammatory neurological diseases, either as adjunctive treatment or as first-line therapy. However, whether IVIg has therapeutic potential on HD remains unclear. Here we for the first time demonstrated that IVIg treatment remarkably rescued motor and cognitive deficits, prevented synaptic degeneration, attenuated neuroinflammation and oxidative stress in R6/2 mouse model. Further investigation showed that the beneficial effects of IVIg resulted from the reduced levels of mutant HTT and inhibition of NF-κB signalling pathway. These findings suggest that IVIg is a promising therapeutic potential for HD.

1. Introduction

Huntington's disease (HD) is a neurodegenerative genetic disorder characterized by progressive motor, cognitive and psychiatric deficits, which has a prevalence of 5–10 cases per 100,000 people worldwide, with regional variations (Schapira et al., 2014). To date, there is no cure or disease-modifying treatment for HD, and only a few HD treatment options were approved by FDA, which provide temporary symptomatic relief to control the motor disorders and psychiatric problems in the early stages.

HD is caused by an extended CAG-repeat tract in exon 1 of the huntingtin (*HTT*) gene which encodes a mutant huntingtin (mHTT) protein with a polyglutamine (polyQ) expansion (Wang et al., 2014). The polyQ repeats are highly polymorphic. Genetic and epidemiological data indicated that 36 or more polyQ repeats were identified to contribute to HD pathogenesis in humans (1993). The polyQ expansion induces a conformational change in mHTT which forms aggregates in both nucleus and cytoplasm of affected neurons (DiFiglia et al., 1997). Multiple lines of evidence indicated that the progressive aggregation of mHTT played important roles in the pathogenesis of HD, including proteasomal dysfunction, transcriptional dysregulation, oxidative stress, mitochondrial and metabolic dysfunction, impaired energy metabolism, abnormal protein-protein interaction, neuroinflammation and eventual cell death (Jimenez-Sanchez et al., 2016; Labbadia and Morimoto, 2013). Several strategies were applied to treat experimental animal models, such as antisense oligonucleotides targeting the CAG repeat region to reduce HTT mRNA expression (Rue et al., 2016), intrabodies or recombinant single chain Fv (scFv) antibody fragments to counteract abnormal protein production (Zha et al., 2016), a small molecule ligand to normalize p75NTR signaling (Simmons et al., 2016a), and phosphodiesterase 10A inhibitor to correct basal ganglia circuitry (Beaumont et al., 2016). However, none of them has been tested in HD patients.

Intravenous immunoglobulin (IVIg) is a preparation of polyclonal serum IgG pooled from thousands of blood donors, and

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has been used for nearly three decades. As an efficient anti-inflammatory and immunomodulatory adjunctive or first-line therapy, IVIg has been used to treat a growing number of autoimmune or inflammatory neurological diseases (Lunemann et al., 2015). Evidences of randomized trials support the effectiveness of IVIg in various acute and chronic demyelinating neuropathies, neuromuscular transmission defects, inflammatory myopathies, as well as in stiff-person syndrome. Moreover, IVIg showed promise for the treatment of various neuroinflammatory-related neurodegenerative disorders (Dalakas, 2014), such as Alzheimer's disease (AD) (Relkin, 2014) and Parkinson's disease (PD) (Patrias et al., 2010; Smith et al., 2012). IVIg played a critical therapeutic role on AD animal model via an immune-mediated Aβ-degradation pathway (Dodel et al., 2004; Wang et al., 2016). Although IVIg failed to effectively treat AD patients in phase III trials, it was superior to placebo in treating apolipoprotein E ε4 carriers (Lunemann et al., 2015). Moreover, IVIg may be a beneficial treatment for PD by reducing α-synuclein oligomer neurotoxicity via specific antibodies against soluble conformations of a-synuclein (Smith et al., 2012). Despite its widespread use and therapeutic successes, the effect of IVIg on HD remains unclear.

A variety of HD mouse models have been developed. The most widely used and best characterized is R6/2, which ubiquitously expresses the 5′ end of the human *HTT* gene carrying only exon 1 with 120 CAG repeat. The R6/2 mice display a progressive degenerative phenotype similar to HD patient with a very early symptomatic onset at 6–8 weeks (Menalled et al., 2009). Here, we assessed the effect of IVIg on the motor symptoms and neuropathological features of R6/2 mouse model.

2. Results

2.1. IVIg attenuated motor and cognitive deficits in R6/2 mice

The R6/2 mice used in this study express exon 1 of human *HTT* with highly expanded CAG repeats (120 ± 5 CAGs), which display rapid symptomatic onset and robust disease progression (Sathasivam et al., 2013). This model exhibits HD-like symptomology with loss of body weight, impaired motor activity, synaptic degeneration and the presence of HTT-positive aggregates in brains. Despite of the increased caloric intake in HD patients (Trejo et al., 2004), weight loss is still a common feature of HD (Aziz et al., 2008), which is also observed in R6/2 mice. Our results showed that vehicle-treated R6/2 mice began to lose body weight at 7 weeks of age, while IVIg treatment inhibited the weight loss, which reaching statistical significance at 9 weeks of age ($p < 0.05$, Fig. 1B).

To assess the effect of IVIg on motor coordination, R6/2 mice and their WT littermates were tested with the accelerating rotarod task at 10 weeks of age. R6/2 mice showed impaired motor performance by falling off the rotarod sooner than their WT littermates. Notably, IVIg-treated R6/2 mice stayed on the accelerating rotarod obviously longer than vehicle-treated mouse controls, showing IVIg's protective effects against motor coordination deficits in R6/2 mice ($p < 0.05$, Fig. 1C). Hindlimb clasping has been observed in various neurodegenerative mouse models, including R6/2 phenotype (Cummings et al., 2012). Clasping rarely occurred in WT mice, whereas R6/2 mice exhibited severe hindlimb clasping behavior at 10 weeks of age. Compared with vehicle treatment, IVIg attenuated hindlimb clasping behavior of R6/2 mice by significantly decreasing the hindlimb severity scores ($p < 0.01$, Fig. 1D).

Besides motor performance abnormalities, cognitive impairment is another important clinical characteristic of HD patient (Doria et al., 2015). The object recognition test was performed to evaluate the effect of IVIg on the cognitive deficits in R6/2 mice. Our results showed that WT mice exhibited a significant increase in the percentage of investigations to the novel object between training and test session in contrast to the R6/2 mouse control. However, IVIg treatment improved cognitive function of R6/2 mice by showing significant preference to the novel object ($p < 0.05$, Fig. 1E).

2.2. IVIg mitigated hypoactivity and anxiety-behavior in R6/2 mice

To assess the effect of IVIg on locomotion, exploration and anxiety-behavior in R6/2 mice, we performed an open field test. Compared with WT mice, R6/2 control mice showed significant hypoactivity, with less distance travelled in total ($p < 0.001$, Fig. 2A) and in the center area ($p < 0.001$, Fig. 2B), as well as less rearings ($p < 0.001$, Fig. 2C). However, IVIg treatment normalized the hypo-locomotion of R6/2 mice, resulting in travelling more distance and rearing more frequently (Fig. 2A-C). Moreover, vehicle-treated R6/2 mice exhibited robust anxiety-behavior by entering the center area infrequently ($p < 0.001$, Fig. 2D) and spending less time in the center area ($p < 0.001$, Fig. 2E), IVIg ameliorated the behavior abnormalities, showing more center entries ($p < 0.001$, Fig. 2D) and longer center duration ($p < 0.001$, Fig. 2E), without the interference with their velocity ($p > 0.05$, Fig. 2F). These findings supported the beneficial effect of IVIg on the improvement of locomotion and psychiatric behavior of R6/2 mice.

2.3. IVIg lowered the levels of mHTT aggregates and oligomers in the brains of R6/2 mice

Progressive accumulation of mHTT in cortex and striatum are main pathological feature of HD (Wang et al., 2008). We performed immunostaining using antibody EM48 to evaluate the effect of IVIg on the mHTT aggregates in R6/2 mouse brains (Fig. 3A). IVIg treatment significantly reduced the total area occupied by mHTT aggregates in the cortex and striatum by 63.4% ($p < 0.001$, Fig. 3B) and 63.3% ($p < 0.001$, Fig. 3C), respectively. The neuronal intranuclear HTT aggregates in the cortex and striatum of R6/2 mice were also detected by EM48 immunofluorescence. The results showed that the total area occupied by intranuclear HTT aggregates in the cortex and striatum of R6/2 mice was significantly reduced by IVIg treatment ($p < 0.01$, Fig. 3D, E).

In the brains of HD patients and HD mouse models, mHTT may pathologically assemble into different types of oligomers, and further aggregate into fibrils (Crook and Housman, 2011). Increasing evidences indicated that the soluble oligomers represented the primary toxic species of amyloids and played a key role in the pathogenesis of amyloidoses (Guerrero-Munoz et al., 2014; Kayed and Lasagna-Reeves, 2013). We next determined the levels of mHTT oligomers in the mouse brain lysates by dot-blot using fibrillar oligomer-specific antibody OC and prefibrillar oligomer-specific antibody A11 (Kayed et al., 2007) (Fig. 3F). IVIg-treated mice showed 63.3% ($p < 0.001$, Fig. 3G) and 42.1% ($p < 0.001$, Fig. 3H) reductions in the levels of mHTT fibrillar and prefibrillar oligomers, respectively, relative to vehicle-treated R6/2 mice. These mHTT-lowered effects of IVIg may contribute to its protective function against motor and cognitive impairments in R6/2 mice.

2.4. IVIg attenuated neuroinflammation in the brains of R6/2 mice

The increased glial activation and inflammatory factor production importantly contributed to HD pathogenesis (Chang et al., 2015; Moller, 2010). The microgliosis and astrocytosis in the mouse brains were detected by Iba-1 and GFAP immunoblotting and immunostaining, respectively. The western blot results showed that IVIg significantly reduced the levels of Iba-1 and GFAP in R6/2 mouse brain lysates by 42.5% ($p < 0.001$, Fig. 4A, B) and