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Intracerebral Transplantation of Neural Stem Cells Combined with Trehalose Ingestion Alleviates Pathology in a Mouse Model of Huntington's Disease

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Abstract

The present investigation examined the neuroprotective benefits for combined trehalose administration with C17.2 neural stem cell transplantation in a transgenic mouse model of Huntington's disease (HD), R6/2. C17.2 neural stem cells have the potential of differentiating into a neuronal phenotype *in vitro* and have been shown to be effective in the treatment of a variety of lysosomal lipid storage disorders in the nervous system. In this study, we transplanted these cells into the lateral ventricle of R6/2 transgenic mice in order to examine the efficacy of using these cells for correcting the accumulated polyglutamine storage materials in HD. To improve efficacy, animals were fed with a diet rich in trehalose, which has been shown to be beneficial to retard aggregate formation. The combined treatment strategy not only decreased ubiquitin-positive aggregation in striatum, alleviated polyglutamine aggregation formation, and reduced striatal volume, but also extended lifespan in the R6/2 animal model. Behavioral evaluation showed that the combination treatment improved motor dysfunction. Statistical analysis revealed that the combination treatment was more effective than treatment with trehalose alone based on the above biochemical and behavioral criteria. This study provides a strong basis for further developing an effective therapeutic strategy for HD.

Keywords

Huntington's disease; neural stem cell; cell transplantation; polyQ; polyglutamine; trehalose

INTRODUCTION

The hallmark events in a number of neurodegenerative diseases, such as Huntington's disease (HD), Alzheimer's disease, the prion encephalopathies, and Parkinson's disease, are changes in the secondary and/or tertiary structure of normal functional proteins. These changes affect protein folding, leading to the formation of misfolded proteins, such as polyglutamine (polyQ)-containing proteins, amyloid β protein, prion protein, tau protein, and α -synuclein. The diseases associated with abnormal protein aggregation are now grouped together as the "protein conformational diseases" (Merlini et al., 2001; Selkoe, 2004). The abnormal deposition of misfolded proteins may take place in caveolae and induce malfunction of a distinctive set of neurons. It may induce oxidative and endoplasmic reticulum stress and proteosomal and mitochondrial dysfunction, which ultimately may lead to neuronal death (Shastri et al., 2003).

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HD is a progressive neurodegenerative disorder characterized by chorea and progressive dementia. The main pathology is a loss of medium spiny projection neurons in the striatum. A mutation in an expansion of CAG repeats in the huntingtin (Htt) gene encoding polyQ leads to the formation of polyQ aggregates in the cells of HD patients (Zoghbi and Orr, 2000). It has been proposed that polyQ aggregates induce endoplasmic reticulum (ER) stress and activates the IRE1-TRAF2-ASK-JNK cell death pathway (Nishitoh et al., 2002). Other studies showed that altered Htt tangles inhibit the function of CREB-binding protein (also known as CBP), a smaller regulatory protein that is essential for cell survival (Nucifora et al., 2001). Htt aggregates pull CBP away from its normal position alongside the DNA in the nucleus. Once seized, CBP can no longer accomplish its normal function of activating transcription for cell survival (Nucifora et al., 2001). Recently, Bence et al. (2001) suggested that altered Htt aggregates impair the function of the ubiquitin-proteasome system (UPS). Protein aggregates may occupy the proteasomes and inhibit their normal activity. At present, it is not clearly understood how Htt bundles can lead to neuronal cell death. More importantly, there has not been any effective treatment for this disease.

The disaccharide trehalose is found in a wide range of mammalian and non-mammalian species (Chen and Haddad, 2004). It has been shown that trehalose can affect protein misfolding/folding and aggregation (Welch and Brown, 1996; Singer and Lindquist, 1998). Trehalose is also known to inhibit the formation of insulin amyloid (Arora et al., 2004) and suppress the aggregation of denatured proteins in yeast (Singer and Lindquist, 1998). More recently, Tanaka et al. (2004) reported that oral administration of trehalose alleviates disease symptoms by reducing the formation of Htt aggregates. Furthermore, trehalose has been demonstrated to enhance a novel mTOR-independent autophagy to accelerate the clearance of mutant Htt and α -synuclein (Sarkar et al. 2007). Thus, trehalose has multiple functions in regulating protein folding, enhancing autophagy, and serving as a molecular chaperone. These studies suggest that trehalose can be a potential therapeutic agent for HD.

In recent years, cell transplantation has received growing attention as a potential restorative therapy for a wide variety of neurodegenerative disorders (Lindvall et al., 2004; Dunnett and Rosser, 2004; Gaillard et al. 2007). Neural stem cells (NSCs) should be an excellent source for cell transplantation in HD; they could participate in normal central nervous system (CNS) development and differentiate into regionally appropriate cell types in response to environmental factors (McKay, 1997; Flax et al., 1998).

C17.2 NSCs were derived from a clonal, multipotent mouse progenitor cell line (Ryder et al., 1990). Synder et al. (1992) successfully immortalized dividing cells from the cerebellum at day 4 using a v-myc oncogene. Several clones were characterized and one of the clones, C17.2, fulfilled the criteria of multipotent stem cells. This cell line has the ability to self-renew, differentiate into neuronal and glial lineages of cells, and populate the degenerating CNS (Snyder et al., 1992, 1997; Rosario et al., 1997; Lee et al., 2007). In addition, the ability of these cells to differentiate appeared to be easily achieved compared to their nonimmortalized cerebellar counterpart when transplanted into the hippocampus, stratum, or cortex. C17.2 cells appeared to be able to respond in a site- and tissue-specific fashion to combine seamlessly into the host environment (Riess et al., 2002; Yang et al., 2002). For this reason, C17.2 cells have been used for transplantation therapy in a variety of neurodegenerative conditions, such as Parkinson's disease (Ryu et al. 2005), traumatic brain injury (Reiss et al., 2002; Boockvar et al. 2005), spinal cord injury (Lu et al., 2003), and Sandhoff's disease (Lee et al. 2007) with varying degrees of success. In our investigation, we evaluated the effects of integrating trehalose administration with C17.2 transplantation on truncated protein aggregation and associated motor dysfunction in the transgenic mouse model of HD, R2/6, with the goal of improving the efficacy of a therapeutic strategy using trehalose alone.

MATERIALS AND METHODS

Cell culture and preparation for transplantation

The C17.2 murine neural progenitor cell line was kindly provided by Dr. Evan Snyder (Burnham Cancer Institute, La Jolla, CA). The cells had previously been endowed with the *lacZ* gene encoding the β -galactosidase protein, detectable by a 5-bromo-4-chloro-3-indolyl β -galactoside (X-Gal) for histochemical examination (Snyder et al., 1997). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and 5% horse serum (HS) at 37°C in a humidified 5% CO₂ atmosphere. Before transplantation, the C17.2 cells were trypsinized, gently triturated in serum-containing medium to inactivate trypsin, and washed three times by gently pelleting the cells at low-speed centrifugation (1,000 rpm, 3 min). The cells were suspended in a final concentration of 2×10^4 viable cells/ μ l with phosphate-buffered saline (PBS, pH 7.4). The suspension was kept on ice and gently triturated before each injection to keep the suspension dispersed and free of cell clumps.

Animals

Heterozygous transgenic R6/2 mice, transgenic for the 5' end of the human HD gene carrying (CAG)₁₁₅-(CAG)₁₅₀ repeat expansions, and wild-type B6CBAF1/J mice were obtained from Jackson Laboratories (Bar Harbor, ME). Genotyping was accomplished by polymerase chain reaction (PCR) analysis of genomic DNA isolated from tail biopsies according to the instructions of Jackson Laboratories. A 0.2% trehalose solution was orally administered to both strains of mice at 3 weeks of age and continued until the day the mice were sacrificed. At 8 weeks of age, groups of both strains of mice underwent cell transplantation as follows. Mice were anesthetized with an intraperitoneal (i.p.) injection of Ketamine HCl (60 mg/kg) and Xylazine (8 mg/kg), then they were placed in a Kopf small animal stereotaxic instrument (David Kopf Instruments, Tujunga, CA). The head was positioned by means of a nose clamp adaptor supplemented by ear bars placed lightly in the external auditory meatus to locate the interaural line. The skin of the skull was carefully sheared and a 1 mm-diameter hole was drilled through the skull. C17.2 cells, in 10 μ l (2×10^4 viable cells/ μ l), were injected into the left lateral ventricle of the experimental animal. After sealing the hole with bone wax and sewing the sheared skin, the animals were monitored for the next 3 days.

Immunocytochemistry and microscopy

Perfused mouse brains were removed from 13-week-old mice and sliced to 2-mm thick slices with a brain blocker (David Kopf Instruments, Tujunga, CA). The slices were fixed in 4% paraformaldehyde for 1 h at room temperature and stored in 30% sucrose overnight at 4°C, then frozen at -70°C for at least 30 min and cryostated into 20 μ m thickness cryosections for Nissl staining and immunocytochemistry. The sections were then incubated with primary antibodies against Nestin (BD Pharmingen, San Jose, CA; 1:200), microtubule-associated protein-2 (MAP-2; Sigma; 1:200), ubiquitin (Dako, Carpinteria, CA; 1:300), or β -actin (Sigma; 1:500) in 0.5% BSA for 60 min at room temperature. Primary antibodies were omitted in negative controls. After washing three times in PBS for 10 min, the sections were stained for 60 min with the appropriate fluorophore-conjugated secondary antibody at 1:300 dilution in PBS against specific light chains of mouse or rabbit IgG (Jackson IR Lab, West Grove, PA). Stained cultures were viewed and photographed under a Zeiss LSM510 confocal laser scanning microscope using appropriate fluorescence filters. The areas of lateral ventricles of the Nissl-stained sections were measured with IMAGEJ 1.32J (National Institutes of Health, Bethesda, MD).

Western blot analysis

Whole brains were removed from 13-week-old mice and homogenized in a lysis buffer containing 1% Triton X-100, 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 2 mM Na₃VO₄, and a protease inhibitor cocktail (Sigma). Protein concentration was determined with a Bio-Rad protein assay kit. Lysates containing equal amounts of proteins were subjected to SDS-PAGE, followed by transfer of the protein bands to a nitrocellulose membrane (Bio-Rad). Western blot analysis was performed using monoclonal antibody (Mab) expanded polyglutamine (1C2) (Chemicon, Temecula, CA; 1:1000) and anti- β -actin (Sigma; 1:1000). Quantitative data were obtained using computing densitometer software ImageQuant 5.0.

Paw clasping

R6/2 mice exhibit a special paw clasping phenotype when suspended by the tail (Mangiarini et al., 1996). Evaluation of the clasping phenotype has previously been used to study disease progression in R6/2 mice (Tanaka et al., 2004; Hickey et al., 2005; Nguyen et al., 2005). The 13-week-old mice were suspended by the tail for 30 s, and the foot-clasping time was scored such that a 0- to 5-s clasping duration was given a score of 1, 5-10 s a score of 2, and >10 s a score of 3.

Body weight

Body weight was measured twice per week.

Footprint test

Evaluation of the footprint test has previously been used to study motor deficit progression in R6/2 mice (Carter et al., 1999). Briefly, to obtain footprints, the hind- and forefeet of the mice were coated with blue and orange non-toxic paints, respectively. Each mouse was placed in a corner of the open field and allowed to walk across the paper, leaving a track of footprints. If a mouse did not walk forward, it was considered to have failed the task. Stride lengths were recorded by measuring the distance of forward movement between each stride. For each step parameter, three values were measured from each run. The mean value of each set of three values was used for analysis.

Behavior test

The Rotarod and Y-maze tests have been previously used to evaluate the motor function and recognition memory, respectively (Carter et al., 1999; Nguyen et al., 2005; Ladurelle et al., 2000; King and Arendash, 2002). Tests for our study were executed by trained personnel at the Small Animal Behavior Core Laboratory at the Medical College of Georgia. Briefly, in the Rotarod test a mouse was placed on a rotating rod. The speed of rotation was gradually increased to 40 rpm, and the mouse's ability to remain on the rotating rod in 5 min was recorded. The apparatus of Y-maze consisted of three identical alleys (40-cm long, 12-cm wide, and 35-cm high) disposed as a regular Y (the three arms make 120° angles between each other). With one arm of the Y-maze blocked, the subject mouse was placed in one of the arms and was allowed to explore the 2 arms for 15 minutes. The experimental mouse was returned to the maze 1-4 hours later with all arms open and was scored for 5 minutes. The first arm entered, the amount of time spent in each arm, and the number of entries into each arm were recorded.

Data Analysis

Each experiment was performed in duplicate and the average time was included for quantification. Data are expressed as the mean $\pm$ S.E.M. of averages from at least three experiments. One-way analysis of variance (ANOVA) was used to assess the statistical

significance of the differences, and a *p* value of less than 0.05 was considered statistically significant.

RESULTS

Effect of combined trehalose administration and C17.2 cell transplantation on reduction of polyQ aggregation in R6/2 mouse brain

Previous studies have demonstrated that C17.2 cells possess the ability to differentiate into neurons and glial cells (Snyder et al., 1992, 1997; Rosario et al., 1997). We first attempted to verify that the C17.2 cells that we used retained this ability. As shown as Fig. 1A, more than 60% MAP-2⁺-positive cells were observed from Nestin⁺-positive C17.2 NSCs within 5 weeks of incubation. The C17.2 cells that were endowed with the *lacZ* gene were injected into the lateral ventricle of 8-week-old mice. After transplant, animals were recovered and observed until 13 weeks of age. As shown in Fig. 1B, we observed significant positive blue stain appearing on both sides of the lateral ventricle, motor cortex, and striatum in the C17.2 transplantation group after staining with X-Gal. In immunocytochemical studies, widely distributed ubiquitin-positive aggregates could be stained with a specific antibody in the striatum of R6/2 transgenic mice (Fig. 1C). Oral administration of trehalose unambiguously reduced aggregation in these mice; however, the combined treatment of trehalose administration and C17.2 cell transplantation significantly diminished aggregation when compared with trehalose administration alone.

Analysis of whole brain lysates from 13-week-old mice using Western blotting probed by a monoclonal antibody against expanded polyQ sequence (1C2) revealed a 2.44-fold higher amount of polyQ aggregation in the brain of R6/2 mice compared to the wild-type mice (Fig. 1D). Trehalose administration alone and the combined trehalose treatment and C17.2 transplant group had reduced polyQ aggregation levels of 1.65-fold and 1.23-fold, respectively. These data demonstrated that C17.2 cells could be successfully integrated into the brain of R6/2 transgenic mice and that the combined treatment with trehalose administration and C17.2 transplantation showed an additional effect in reducing ubiquitin-positive polyQ aggregation in the mutant mice.

Striatal volume analysis

The major sites of cell loss in HD brains are the striatum and the cortex (Storey et al., 1992) and in R6/2 mice, striatal atrophy is reflected by expansion of the size of the lateral ventricles. Striatal volume was quantified bilaterally in Nissl-stained coronal sections in all mice (Fig. 2A). The average area of lateral ventricle of R6/2 mice was $0.884 \pm 0.083 \text{ inch}^2$. Treatment with trehalose alone or with combined trehalose administration and C17.2 transplantation significantly reduced the area to $0.334 \pm 0.021 \text{ inch}^2$ and $0.159 \pm 0.027 \text{ inch}^2$, respectively (Fig. 2B), and the difference between these two groups was highly significant (*p* = 0.012). The result showed the combination treatment group had a better neuroprotection effect than the trehalose administration alone group.

Analyses of phenotype, clasping score, body weight, and survival rate

Foot clasping, a dystonic posturing of the hind limbs on suspension by the tail (Fig. 3A), is a characteristic behavior during the later stages of the disease in R6/2 (Mangiarini et al., 1996). Oral administration of 2% trehalose clearly delayed the onset and frequency of the foot-clasping posture and decreased the average clasping score at 13 weeks from 2.875 ± 0.142 (of 3 maximum) to 1.519 ± 0.212 , a 47.2% reduction (Fig. 3A, B). The combination treatment group exhibited a better inhibitory effect than the trehalose alone group - the average clasping score was reduced to 1.180 ± 0.154 (Fig. 3B). With respect to weight changes, the weight gain in R6/2 mice diverged from the wild-type mice and reached a

plateau between six to eight weeks, which was followed by a steady weight loss (Fig. 3C). With respect to the treatment groups, the weight gain in R6/2 was comparable to that of the vehicle-treated group to the eighth week of age but trended toward a gain thereafter, with weights greater than for the untreated animals (Fig. 3C). The average lifespan of vehicle-treated animals was 103.2 ± 2.1 d (Fig. 3D). Trehalose treatment extended the lifespan to 117.3 ± 2.2 d. However, 47.3% of the mice undergoing the combination treatment had an extended lifespan of 140 d as shown in Fig. 3D.

Evaluation of motor function and memory performance improvement

We next employed the footprint, Rotarod, and Y-maze tests to evaluate the motor function and memory performance improvement for the treatment strategies. Gait abnormalities were assessed by analyzing the footprint pattern of mice while they walked along a narrow corridor. As shown as Fig. 4A, wild-type mice, 13 weeks old, walked in a straight line, with a regular evenly alternating gait. By contrast, R6/2 mice weaved from side to side while walking along the corridor, adopting unevenly spaced shorter strides and staggering movements. The trehalose-alone group improved the alternating gait to an even more regular gait and increased the stride length; however, some scuffed gait was observed (Fig. 4A, B). The combined treatment group not only increased the average stride length, but also improved the scuffed and staggering movements, showing that the combination treatment strategy improved the walking posture of the mice.

In the Rotarod test, R6/2 mice at 9 weeks of age showed non-significant maintenance of balance on the Rotarod when compared with wild-type mice (Fig. 5A). With increasing age, R6/2 mice, 13 weeks old, significantly reduced the lasting time from 77.10 ± 4.35 s to 29.39 ± 2.71 s. The trehalose-alone group and the combination treatment group had increased lasting times on the Rotarod, to 38.09 ± 4.54 s and 47.17 ± 2.35 s, respectively (Fig. 5A). Statistical analysis performed by ANOVA showed a significant difference between the combination group compared with the trehalose alone group ($p < 0.01$).

The Y-maze test is based on the interest of mice for novelty; hence, they explore preferentially unknown territories. The number of explorations of each arm was counted and the percentage of visits calculated. As shown as Fig. 5B, significantly fewer numbers of entry in 9-week-old R6/2 mice with or without treatment was observed. In 13-week-old R6/2 mice, the vehicle-treated control group still had lower entry numbers and percentages, whereas the combination-treatment group showed a significantly increased number of entries than the control group ($p < 0.01$) (Fig. 5B). These results indicate that a combination of trehalose administration and C17.2 transplant strategy significantly improved motor function and memory performance in R6/2 mice.

Taken together, the combined treatment strategy clearly showed significant neuroprotection and improvement of motor functions as evidenced by reduced ubiquitin-positive, polyQ aggregation in striatum, lowered lateral ventricle area, prevention of body weight loss, extension of lifespan, and improved motor function in the R6/2 mice model. Comparing with the trehalose-administration alone group, the combination strategy also showed better survival rate and motor function.

DISCUSSION

In this study, we investigated whether combined trehalose administration with C17.2 NSC transplantation strategy have improved efficacy compared to using trehalose administration alone in the treatment of HD symptoms in a mouse model of HD. Our goal was to examine if cell transplantation could enhance the efficacy of trehalose treatment. Our results clearly showed that C17.2 NSCs, when transplanted into the brain, retained the ability to

differentiate into neurons. The transplanted cells could enhance the efficacy with trehalose treatment alone to reduce ubiquitin-positive and polyQ aggregation. This combination treatment strategy significantly increased the lifespan and reduced motor dysfunction in the R6/2 transgenic mice model.

NSCs have been shown to be a promising agent for cell replacement and gene transfer therapy in neurodegenerative diseases (McKay, 1997; Flax et al., 1998; Fukuhara et al. 2006; Lee et al., 2007). Although the brain is remarkably adaptable in tolerating donor stem cells from even a different species (Jeong et al. 2003; Meng et al. 2003), we chose C17.2 cells because the most robust results have been obtained with this cell line. C17.2 cells have illustrated several fundamental biological processes such as directed migration (Snyder et al., 1992) and homing (Liu et al., 1999) and have provided direct demonstration of the ability of the brain to direct site- and region-specific differentiation and repair (Lu et al., 2003; Riess et al., 2002). Previous studies demonstrated that a C17.2 NSC transplantation strategy had significantly improved neurological motor functions in certain neurodegenerative models, such as Parkinson's disease (Ryu et al., 2005), traumatic brain injury (Riess et al., 2002; Boockvar, et al., 2005), and spinal cord injury (Lu et al., 2003). The major questions derived from these transplant strategies are: 1) Can they produce neurons? and 2) Can they survive transplantation into animal models of HD and effect circuit reconstruction and restore lost function? Snyder's study showed the differentiation fate of the C17.2 NSCs was distinctly different in regions undergoing targeted apoptotic neuronal degeneration compared with control environments (Snyder et al., 1997). In intact adult neocortex, the transplanted precursors either differentiate into glia or remain undifferentiated. However, within regions of adult neocortex undergoing targeted neuronal degeneration, significantly more precursors have differentiated into neurons by the same criteria. These studies showed a variety of molecular and cellular signals are involved in the differentiation of C17.2 NSCs. Our results clearly demonstrate that C17.2 cells could differentiate into neurons, consistent with previous reports (Snyder et al., 1992; 1997; Lee et al., 2007), and presence of positive X-Gal stains in the lateral ventricles clearly demonstrated that the engrafted C17.2 cells survived after transplantation. These results are consistent with previous observations that C17.2 NSCs may provide an alternative cell source for transplant therapy for the lesion areas.

PolyQ aggregation has been identified as playing an important role in the pathogenesis of HD (Sanchez et al., 2003), and inhibition of aggregation can lead to amelioration of polyQ-mediated pathology (Bates, 2003). Previous studies revealed that trehalose increased the stability of a polyQ-containing protein made the protein more resistant to proteolysis by caspases (Ona et al., 1999; Chen et al., 2000; Sanchez et al., 2003) because polyQ-containing Htt is a substrate for caspases -2, -3 and -6 (Hermel et al., 2004; Cajavec et al., 2005). In addition, Chu et al., (2003) showed that C17.2 NSCs constitutively secrete neurotrophic factors such as glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF). GDNF can regulate the levels of certain inhibitors of apoptosis (IAP) to inhibit caspase activities (Perrelet et al., 2002). Topical application of GDNF on the brain surface potentiates and prolongs *p*-Akt activation, but suppresses activation of the caspases, and reduces the numbers of TUNEL-positive cells (Jin et al., 2005). In the present context, a reasonable speculation that combined treatment showed more prominent effect than trehalose treatment alone in reducing polyQ aggregation may be through the releasing of neurotrophic factors from the transplanted C17.2 NSCs. These factors reduced the activation of caspases, increased the stabilizing ability of trehalose in polyQ-containing proteins. Clearly, further investigations are needed to clarify if any of the mechanisms may play a dominant role. Regardless of the detailed mechanisms, our results clearly suggest the beneficial effects of a combined trehalose administration with a C17.2

NSC transplantation strategy in reducing brain atrophy, improving motor dysfunction, and extending the lifespan of R6/2 transgenic mice.

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Abbreviations

HD	Huntington's disease
polyQ	polyglutamine
Htt	huntingtin
CBP	CREB-binding protein
UPS	ubiquitin-proteasome system
NSCs	neural stem cells

Fig. 1. Effects of trehalose and trehalose combined with C17.2 NSC transplantation treatment on polyQ-mediated pathology. (A) C17.2 cells were analyzed by immunocytochemistry before and after 5 weeks of culture *in vitro*, by double labeling of Nestin (green; 1:200) and MAP-2 (red; 1:200). Stained cultures were viewed and photographed under a Zeiss LSM510 confocal laser scanning microscope using appropriate fluorescence filters. (B) Perfused mouse brains were removed from 13-week-old mice that received vehicle or C17.2 transplantation, chopped to 2-mm thickness slices with a brain blocker and processed for X-gal stain. Arrows means X-gal stain positive area. (C) Striatum slices from 13-week-old wild-type or R6/2 transgenic mice that received trehalose administration or combined

trehalose with C17.2 NSC transplantation marked with ubiquitin (red; 1:300) or β -actin (green; 1:500) were observed by confocal laser scanning microscope. (D) Western blot analysis of whole brain from wild-type or R6/2 mice receiving different treatments as indicated were done with Mab polyQ (1:1000) or β -actin (1:1000). Quantitative data that obtained by densitometry were labeled. Scale bar, 50 μ m.

Fig. 2. Reduction in striatal atrophy in mouse brain. (A) Nissl-stained frozen sections of cerebrum from 13-week-old wild-type or R6/2 mice received different treatments as indicated. Phase contrast photomicrographs were taken by a Zeiss AxioCam digital color camera. (B) Striatal volume was analyzed by IMAGEJ 1.32J software. $n = 3$ for each treatment group. Bars and error bars represent mean $\pm$ SEM. *, $p < 0.05$. Scale bar, 50 μ m.

Fig. 3. Effects of behavior, weight, and survival *in vivo*. Bars and error bars represent mean $\pm$ SEM. (A) Illustration of clasp behavior. 13-week-old wild-type or R6/2 mice with different treatments exhibited clasp. (B) Clasp scores were analyzed at 13 weeks of age in different treatment of wild-type or R6/2 mice. $n = 6$ for each group. *, $p < 0.05$ as compared with wild-type group; †, $p < 0.05$ as compared with R6/2 mice without treatment group. (C) Body weight over time of different groups as labeled. $n = 6$ for each group. (D) Kaplan-Meier analysis of lifespan in different groups.

Fig. 4. Representative walking footprint patterns of mice. (A) The footprint of 13-week-old wild-type or R6/2 mice with different treatments as indicated were recorded. Orange and blue prints represent fore- and hind-paws respectively. (B) Stride length of prints were quantitatively analyzed. Bars and error bars represent mean $\pm$ SEM. $n = 3$ for each group. *, $p < 0.05$ as compared with wild-type group; †, $p < 0.05$ as compared with R6/2 mice without treatment group.

Fig. 5.

Behavior evaluations of the mice. (A) Motor coordination on the Rotarod was measured in 9-week- and 13-week-old wild-type or R6/2 mice with the indicated treatment. (B) Y-maze analysis composed of arm entries and percent of novel arm entries over a 5-min trial. Values are mean $\pm$ SEM. $n = 3$ for each group. *, $p < 0.05$ as compared with wild-type group; †, $p < 0.05$ as compared with R6/2 mice without treatment group; #, $p < 0.05$ as compared with different groups as indicated.