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Research Paper

Preconditioning mesenchymal stem cells with the mood stabilizers lithium and valproic acid enhances therapeutic efficacy in a mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by CAG repeat expansions in the huntingtin gene. Although, stem cell-based therapy has emerged as a potential treatment for neurodegenerative diseases, limitations remain, including optimizing delivery to the brain and donor cell loss after transplantation. One strategy to boost cell survival and efficacy is to precondition cells before transplantation. Because the neuroprotective actions of the mood stabilizers lithium and valproic acid (VPA) induce multiple pro-survival signaling pathways, we hypothesized that preconditioning bone marrow-derived mesenchymal stem cells (MSCs) with lithium and VPA prior to intranasal delivery to the brain would enhance their therapeutic efficacy, and thereby facilitate functional recovery in N171-82Q HD transgenic mice. MSCs were treated in the presence or absence of combined lithium and VPA, and were then delivered by brain-targeted single intranasal administration to eight-week old HD mice. Histological analysis confirmed the presence of MSCs in the brain. Open-field test revealed that ambulatory distance and mean velocity were significantly improved in HD mice that received preconditioned MSCs, compared to HD vehicle-control and HD mice transplanted with non-preconditioned MSCs. Greater benefits on motor function were observed in HD mice given preconditioned MSCs, while HD mice treated with non-preconditioned MSCs showed no functional benefits. Moreover, preconditioned MSCs reduced striatal neuronal loss and huntingtin aggregates in HD mice. Gene expression profiling of preconditioned MSCs revealed a robust increase in expression of genes involved in trophic effects, antioxidant, anti-apoptosis, cytokine/chemokine receptor, migration, mitochondrial energy metabolism, and stress response signaling pathways. Consistent with this finding, preconditioned MSCs demonstrated increased survival after transplantation into the brain compared to non-preconditioned cells. Our results suggest that preconditioning stem cells with the mood stabilizers lithium and VPA before transplantation may serve as an effective strategy for enhancing the therapeutic efficacy of stem cell-based therapies.

1. Introduction

Stem cell-based therapy has recently emerged as a feasible therapeutic approach for the treatment of neurodegenerative diseases and may be effective in alleviating the pathophysiological mechanisms of Huntington's disease (HD). One of the major mechanisms for promoting repair is the upregulation of local growth factors and neurotrophins. In this regard, multipotent adult stem cells such as mesenchymal stem cells (MSCs) may offer a potential therapeutic avenue to treat HD since they have the ability to produce trophic factors that activate endogenous neurorestorative processes within the injured brain (Mahmood et al., 2004). MSCs function to secrete bioactive molecules such as chemokines, cytokines, growth factors, gene products, and immunomodulatory peptides that act as signaling molecules which elicit recovery by promoting neurogenesis, angiogenesis, and synaptogenesis (Walker et al., 2009; Xiong et al., 2010). These biological actions support the notion that the short-term beneficial effects garnered from MSC transplantation may not result directly from cell replacement. Instead, the positive effects from transplanted MSCs on increasing the viability of surviving cells may be attributed to indirect effects mediated by secreted beneficial factors that may protect against neuronal death,

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recruit endogenous stem cells, reduce inflammation, and stimulate neurogenesis in the brain. Thus, MSCs may be an attractive cell therapy for targeting HD.

One of the major challenges for using stem cells to treat central nervous system (CNS) disorders includes the identification of an effective delivery strategy to the brain. Although previous studies have shown that systemic administration of stem cells confers beneficial effects to the brain, it is well-established that many of the cells become entrapped in vascular beds of the lungs, liver, kidney and spleen, thus, resulting in few cells actually reaching the brain (Costantino et al., 2007; Dhuria et al., 2010; Mahmood et al., 2004; Wei et al., 2013). To enhance the therapeutic potential of stem cells and avoid unwanted systemic effects, intranasal cell administration may have the potential for achieving targeted CNS delivery. Intranasal delivery is a non-invasive method that bypasses the blood-brain barrier and directly targets therapeutics to the brain. Overall, this delivery method is clinically attractive since it is less invasive than neurosurgical implantation. A range of therapeutics including macromolecules, drugs, or cells can be delivered, and are distributed throughout the entire brain (Chapman et al., 2013; Lochhead and Thorne, 2012; Mittal et al., 2014). Mechanistically, therapeutics reach the brain via the rostral migratory stream, trigeminal nerve, and olfactory nerve (Scranton et al., 2011; Xiao et al., 2013). Recent studies have shown that intranasal delivery of stem cells exerts therapeutic effects in animal models of Parkinson's disease, hypoxia-ischemia, and autoimmune encephalomyelitis (Danielyan et al., 2011; van Velthoven et al., 2010, 2014; Wei et al., 2013; Wu et al., 2013). In addition, neural stem cells and MSCs delivered by intranasal administration were shown to migrate towards and target malignant intracerebral gliomas with therapeutics (Balyasnikova et al., 2014; Reitz et al., 2012). Taken together, these studies provide compelling evidence that intranasal delivery of stem cells is feasible and may be useful as a treatment modality for CNS pathological conditions.

Substantial donor cell loss after transplantation is another challenge facing the field of stem cell-based therapy. Following transplantation into the host brain, stem cells encounter a harsh tissue microenvironment. One strategy to boost cell survival is to precondition stem cells prior to transplantation with compounds or conditions that can enhance their survival and overall therapeutic efficacy. There is considerable evidence that preconditioning provides a therapeutic advantage to stem cells prior to transplantation. Several studies have demonstrated that subjecting stem cells to preconditioning stimuli such as short-term exposure to hypoxia or treatment with pharmacological agents can elicit therapeutic benefits against injuries in the brain and heart (Sakata et al., 2012; Sun et al., 2014; Wei et al., 2012; Xu et al., 2013).

Emerging evidence supports the notion that the mood stabilizers lithium and valproic acid (VPA) exhibit neuroprotective and neurotrophic properties. Lithium and VPA inhibit glycogen synthase kinase-3 (GSK-3) and histone deacetylases (HDACs), respectively, to regulate multifaceted signaling pathways and induce a large number of neuroprotective and neurotrophic proteins (for review see Chiu et al., 2013). Studies from our laboratory and others have shown that lithium and VPA exert robust beneficial effects in diverse preclinical models of neurological and neuropsychiatric diseases (Chiu and Chuang, 2010; Chiu et al., 2013; Chuang et al., 2009). In this regard, the mood stabilizers lithium and VPA may be effective agents to use for preconditioning. Therefore, in the present study we sought to determine the therapeutic benefits of preconditioning MSCs with lithium and VPA prior to intranasal delivery in a transgenic mouse model of HD.

2. Materials and methods

2.1. Experimental animals

Heterozygous male Huntington's disease mice from the N171-82Q strain were obtained from the Jackson Laboratory (Bar Harbor, ME). Male transgenic mice were bred with wild type (WT) females from their background strain B6C3F1/J as described (Chiu et al., 2011). N171-82Q HD mice and their corresponding WT littermate controls were used in the behavioral tests and immunohistochemistry studies. The groups in the study consisted of a combination of male ($n = 28$) and female ($n = 32$) mice. Each group was comprised of 7 males and 8 females. All animals underwent baseline behavioral assessments one week prior to intranasal cell administration and were placed into balanced cohorts based on their performance. Treatment began in 8-week old mice (males and females) and included four recipient groups: 1) WT mice administered PBS vehicle ($n = 15$); 2) N171-82Q HD mice administered PBS vehicle ($n = 15$); 3) N171-82Q HD mice administered MSCs ($n = 15$); and 4) N171-82Q HD mice administered lithium and VPA-treated mesenchymal stem cells ($n = 15$). The animals were housed in cages under a standard 12 h light/dark cycle with food and water available ad libitum. Body weight was measured bi-weekly. All procedures were approved by the Animal Care and Use Committee of the National Institute of Mental Health at the National Institutes of Health.

2.2. Cell culture

Cryopreserved Gibco® C57BL/6 mouse mesenchymal stem cells (MSCs) derived from bone marrow were obtained from Invitrogen Life Technologies (Carlsbad, CA). The cells were characterized with specific MSC surface markers positive for CD29, CD34, CD44, and Sca-1 and negative for CD117 by the manufacturer. These cells were also plastic adherent and demonstrated multipotentiality as demonstrated by the ability to differentiate into osteogenic, adipogenic, and chondrogenic lineages. Bone marrow-derived MSCs were grown in standard growth media consisting of DMEM/F-12 GlutaMAX™ + 10% FBS + 5 μg/ml gentamicin (Invitrogen Life Technologies). Cultures were maintained at 37 °C in a humidified incubator containing 5% carbon dioxide. Cells from passage 4–7 were used for intranasal transplantation. Differences in morphology, proliferation, and MSC surface marker expression were not observed in these cells.

2.3. In vitro preconditioning of MSCs

Mouse MSCs were treated with lithium chloride and VPA (Sigma-Aldrich, St. Louis, MO) or vehicle control as previously described (Tsai et al., 2010, 2011). Briefly, the preconditioning paradigm consisted of treating MSCs with lithium chloride for 24 continuous hours. VPA was added to the culture for 3 h (from hours 18–21) followed by acute washout. Notably, we have previously shown that it was necessary to shorten VPA treatment because prolonged VPA exposure caused apoptosis of MSCs. Cultures were replenished with fresh media that contained lithium chloride during the final three hours of incubation. Following the preconditioning paradigm, MSCs were harvested for subsequent experiments that included intranasal administration or gene expression studies.

2.4. RNA extraction

Total RNA was extracted from MSCs using Trizol and isolation was accomplished using an RNeasy mini kit according to the manufacturer's instructions (Qiagen, Valencia, CA). Following extraction, the RNA samples were DNase-treated with a DNA-free kit (Ambion ® Invitrogen Life Technologies) to remove any residual DNA. RNA quality was assessed with an Agilent Bioanalyzer (Palo Alto, CA) and the RNA concentration was determined using Nano Drop spectrophotometer (Wilmington, DE).

2.5. Microarray analysis of MSCs

A whole genome microarray analysis was performed by MoGene, LC (St. Louis, MO) on vehicle-treated ($n = 4$) and lithium-VPA treated