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Research Report

Transplants of neurosphere cell suspensions from aged mice are functional in the mouse model of Parkinson's

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Abstract

Neural stem cell therapy has the potential to treat neurodegenerative disorders. For Parkinson's disease (PD), the goal is to enhance the dopamine system sufficiently to restore the control of movement and motor activities. In consideration of autologous stem cell therapy for PD, it will be necessary to propagate the cells in most cases from aged brain tissue. We isolated cells from the subventricular zone (SVZ) in the brains of 1-year-old enhanced green fluorescent protein (GFP) mice and generated neurospheres in culture. Neurospheres yielding high numbers of neurons and astrocytes "de novo" were selected and cryopreserved before evaluating the efficacy of neurosphere cell suspensions transplanted to the 6-hydroxydopamine (6-OHDA) model of PD. In mice unilaterally lesioned with 6-OHDA, transplants of neurosphere cell suspensions to the striatum yielded astrocytes and tyrosine hydroxylase positive neurons that reduced or reversed the drug-induced behavioral circling response to amphetamine and apomorphine. Control mice without the cell suspensions showed no change in the motor behavior. Our results indicate that the SVZ in the aged mouse brain contains cells that can be expanded in the form of neurospheres, cryopreserved, re-expanded and then transplanted into the damaged dopamine system to generate functional cell progeny that offset the motor disturbances in the nigrostriatal system.

Theme: Development and regeneration
Topic: Transplantation

Keywords: Parkinson's disease; Mouse; Stem cell; Transplant; Behavior; Cryopreservation

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder with clinical symptoms manifesting after at least 60% of the dopamine neurons in the midbrain have degenerated [1–3]. The loss of the dopaminergic input to the striatum produces the classical symptoms of tremor, bradykinesia, rigidity, and eventually dementia. Alternatives to conventional pharmacological (L-DOPA) therapy are necessary as most patients enter a "wearing off" stage after approximately 6 years of treatment [18]. The management of PD in advanced patients is a complicated problem. When medication becomes ineffective and the side effects increase, surgical procedures including the pallidotomy may be used [10]. Numerous clinical trials with selected cells for therapy have been conducted on patients with advanced Parkinson's [3]. Modest outcomes continue to place cell therapy at the experimental level. Stem cells from embryonic and adult sources are now a strong focus for cell therapy with advantages of self-renewal for expansion and a wide differentiation potential. These features have been used to tag stem cells with great potential as therapeutic tools.

With the knowledge that neural stem cells are present in the nervous system throughout the entire life span [13,19,26,27,40], the potential is real for the isolation of

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stem cells from the aged brain, perhaps even in a degenerative state. The ability to extract adult stem cells from an aging brain is a real possibility especially in view of autologous therapeutics. There are very few reports of stem cells producing functional effects after transplantation [4,21,42]. The cellular and functional outcomes of stem cells recovered from the aged brain and then transplanted into the CNS have not been reported in animal models of neurodegenerative disease.

The 6-OHDA animal model of PD, introduced by Ungerstedt in 1968 is still extensively used to mimic features of dopamine deficiency [39]. 6-OHDA is a selective neurotoxic for catecholaminergic neurons [5,34]. The 6-OHDA auto-oxidative toxic products undergo retrograde axonal transport and destroy the midbrain DA neurons [16]. Verification of the dopamine lesion with dopamine agonists upsets the motor behavior and causes a rotational pattern that has been extremely valuable to assess functional repair of the nigrostriatal system [25,35].

In this study, we show that stem cells can be isolated from the brains of aged mice and expanded as neurospheres. The spheres can be frozen, thawed, and re-expanded to form new neurospheres. Subsequent transplantation of these spheres into the striatum establishes functional motor effects in the mouse model of PD.

2. Materials and methods

2.1. Experimental animals

Adult female C57BL/6J mice (Charles River Laboratories, Montreal) were housed in the McMaster University Central Animal Facility according to guidelines set out by the Canadian Council for Animal Care. All experiments were approved by the Animal Research Ethics Board of McMaster University. Wild type mice and mice with the ubiquitous expression of GFP were used. This transgenic mouse line with the GFP cDNA is under control of a chicken beta-actin promoter and a cytomegalovirus enhancer. The mice have a C57BL/6J genetic background and were made by Dr. M. Okabe of Osaka University, Japan.

2.2. Generation of neurospheres

The procedure used to isolate neurospheres in culture adhered closely to the methods described by Weiss and colleagues [33,40]. Adult C57BL/6J transgenic donor mice (1 year old) carrying the GFP were euthanized with CO_2. The brain was dissected to remove a thin layer of tissue containing the subventricular zone (SVZ) just outside the lateral ventricles. We selected the mice at 1 year because these genetically modified mice are in poor health or do not live past 18 months. The tissue was digested in a mixture of kynurenic acid, trypsin, and hyaluronidase at 36 °C for 90 min. Ovomucoid was used to stop the digestion and the cell suspension was then transferred to serum-free media (DMEM/F12) containing the hormones and supplements listed by Weiss and colleagues [40]. B27 growth supplement (2.0 ml) (Life Technologies, Burlington, ON), 20 μl of epidermal growth factor (Sigma St. Louis, MO), 20 μl of basic fibroblast growth factor (Sigma, St. Louis, MO), and 7.0 μl of heparin (Sigma, St. Louis, MO) were also added to every 100 ml of media. The cells were plated and cultured in 24-well plates at 37 °C in 5% CO_2 and 95% air. The neurospheres generated from this procedure were passed weekly (to a maximum of 5 times) by dissociating the spheres with a combination of the enzymes listed above and mechanical trituration with a Pasteur pipette. After determining the differentiation potential of the neurospheres, the spheres were cryopreserved in the tissue culture media containing 15% dimethyl sulfoxide in 1.8-ml cryogenic vials. The vials were initially frozen to −80 °C before immersion in liquid nitrogen. We cryopreserved the spheres for 1–3 months in this study.

2.3. Nigrostriatal lesions

6-Hydroxydopamine (Sigma, St. Louis, MO) was prepared fresh for each surgery at a concentration of 15 μg in 2.0 μl of 0.02% ascorbic acid dissolved in saline. Stereotaxic injections of 6-hydroxydopamine were performed in 2- to 3-month-old C57BL/6J wild type mice (no GFP expression) under a weight-dependent intraperitoneal injection of ketamine/xylazine (0.1 ml/10 g body weight). Each animal received 1.0 mg/kg buprenorphine for analgesia. We found the lesions to be most extensive and consistent in 2- to 3-month-old mice. The mice were positioned in a stereotaxic frame and the skin was opened along the midline of the skull and a small burr hole was made with the tip of a 30-gauge needle. A 10-μl beveled flexi-tip syringe (World Precision Instruments, Sarasota, FL) was used to administer 2.0 μl of 6-OHDA into the left striatum at a rate of 0.5 μl/min. The coordinates for the unilateral injection were +0.5 mm antero-posterior (AP), 1.5 mm medial–lateral (ML), and 2.5 mm dorso-ventral (DV) from bregma and the skull surface [12]. The mice were allowed to recover for 1 week before behavioral testing. We also used TH immunocytochemistry to verify the extent and the stability of the lesion in the animals.

2.4. Apomorphine and amphetamine behavioral testing

The success of the 6-OHDA lesion was verified with apomorphine and amphetamine. One week post-lesion, the mice received a subcutaneous injection of 0.3 ml of apomorphine (0.6 mg/kg, Sigma, St. Louis, MO). Each mouse was placed in a bowl (24 cm diameter) with fresh bedding and allowed to habituate for 5 min. The number of contralateral turns was scored during the next 5 min.

Two weeks post-lesion, the mice received an intraperitoneal injection of 0.3 ml of amphetamine (10 mg/kg) and