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Transplantation of Adult Bone Marrow Stem Cells Enhances Behavioral Recovery Following a Traumatic Brain Injury (TBI)

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**ABSTRACT**  A potential treatment for millions of new cases of traumatic brain injury (TBI) may be through the transplantation of genetically modified adult bone marrow stem cells (BMSC) in the form of neurospheres. This study examined behavioral recovery in the controlled cortical impact (CCI), a rodent model of TBI, after neurosphere transplantation. Rats received a unilateral CCI over the forelimb sensorimotor cortex. Seven days post-CCI, neurospheres or vehicle control were injected within the cortex or striatum. Forelimb deficits were assessed with two behavioral tests for two months. The test results indicated striatal neurosphere transplants significantly reduced deficits in both behavior tests while other transplantation combinations only resulted in enhancements within either behavioral test or injection site. Results suggest that neurosphere transplantation may be effective in enhancing behavioral recovery following TBI and striatum transplantation may provide a more optimal transplant site than cortex.

**INTRODUCTION**

Traumatic Brain Injury (TBI) affects 1.7 million people annually and is a persistent concern in clinical settings with detrimental neurological and behavioral side effects (Faul et al. 2010). Currently there are no approved treatment options for physicians to administer after a TBI. Yet, there are three known theoretical approaches to the treatment of TBI: the use of behavioral rehabilitation, the reduction of secondary traumatic factors, and the restoration of lost tissue (Gnegchi & Melo 2009). A potential solution is to ameliorate the loss of tissue with transplanted stem cells. Transplantation of different types of cells and tissues has been attempted in several disease conditions, including Parkinson’s disease, stroke, and TBI (Opydo-Chanek 2007).

In the present study, the researchers used mesenchymal stem cells isolated from adult rat bone marrow (BMSCs). The benefits of using adult stem cells include: 1) avoiding ethical ramifications as seen with embryonic cells, 2) diminishing rejection of the transplanted cells from the body, and 3) decreasing use of immunosuppressive drugs. The BMSC are multi-potent meaning they can differentiate into a variety of cell types including osteoblasts, chondrocytes and adipocytes (Gnegchi & Melo 2009). In addition, BMSCs can be transformed into neuronal precursors (immature cells that are destined to become cells of the nervous system) by genetic modification using the intracellular domain of Notch1 and neo-resistance gene (Yasuhara 2009; Hayase 2009). Within culture, hundreds of neuronal precursor cells have affinity to attract and form spheres called neurospheres. The majority of cells within neurospheres derived from Notch-induced BMSCs express neuroprogenitor marker nestin. Neuroprogenitor cells are destined to become neurons. Notch-induced BMSCs have been transplanted into the 6-OHDA rat model of Parkinson’s disease where they have demonstrated a decrease in the death of host neurons.
neurons (Glavaski-Joksimovic et al. 2009). It is thought that the stem cell transplantation may replace the damaged cells or provide a source of growth factors or neuroprotective agents to damaged cells (Opydo-Chanek 2007). While studies using animal models of TBI have showed survival and integration of transplanted BMSC into the neural circuitry, few studies have observed the effects of neurosphere transplantation (Li 2009).

Using the controlled cortical impact (CCI) model of TBI, researchers injected neurospheres one week post-injury into the injured cortex (posterior to the injury) or within the dorsolateral striatum (area connected to the injured cortex). When examined one week post-transplant (two weeks post-injury), the transplanted neurospheres survived and recovery of forelimb function was enhanced suggesting cells transplanted had an effect on function (Favory et al. 2010). The current study attempts to examine the long term effects of transplanting these neurospheres on behavioral function.

**METHODS**

All protocols involving animals were approved by the Institutional Animal Care and Use Committee (IACUC). Animals: Male Fisher 344 rats (250-300g) were used. Rats were kept in the DePaul University Research Facility on a 12:12 light dark cycle and fed ad libitum. Prior to the delivery of the CCI, rats were tested on all behavioral tests for baseline. Animals were randomly chosen and assigned to the following experimental and control groups: CCI only, CCI+Neurospheres (NS) in Vehicle-Cortex, CCI+Vehicle only–Cortex, CCI+NS in Vehicle-Striatum, CCI+NS in PBS–Striatum, CCI+Vehicle only–Striatum, and CCI+PBS only–Striatum (See Table 1).

**Delivery of CCI**: Once anesthetized with isoflurane, animals were placed on a stereotaxic apparatus and received a 4mm diameter craniotomy at 0.5mm anterior and 4mm lateral to bregma. A unilateral CCI was administered over the forelimb sensory motor cortex (FL-SMC). The impact was delivered by the Benchmark Impactor. The impactor was angled 18° away from vertical, placing the flat impactor tip (3mm) perpendicular to the surface of the brain. Once in place, the impactor tip compressed the exposed brain at 3.0 m/s at a depth of 1.7mm below the cortical surface for 300ms. After the impact, the wound was sutured and topical analgesics and antibiotics were applied.

**Creation and Transplantation of Neurospheres**: Neurospheres were received from our collaborators and produced using protocol from Glavaski-Joksimovic (2009). Bone marrow stem cells were harvested from Fischer 344 tibia or femur bone marrow. Cells were cultured in Petri dishes with basal medium and transinfected with Notch1 and neo-resistant genes. Cell cultures were observed to demonstrate spherical qualities of a neurosphere.

Transplantation surgery occurred seven days post CCI to the FL-SMC. Rats were anesthetized with isoflurane and placed in a stereotax prior to exposing the injury site and craniotomy. Locations of transplant injections were determined using rat brain atlas coordinates (See Figure 1). Injured rats received two injections, in two separate locations, into either the injured cortex or dorsolateral striatum ipsilateral to the injury (2µl injections of 25,000/µl, totaling 100,000 cells/animal). Vehicle consisted of neurobasal medium supplemented with B27, Fibroblast Growth Factor, and Endothelial Growth Factor. PBS controls received sterile PBS. There were no injections contralateral to the injury. Injections of neurospheres in PBS acted as control to neurospheres alone contributed to behavioral enhancement.

**Behavior Testing**: Rats were tested on measures of forelimb motor function following injury and transplantation. Tests were conducted on days 0, 5, 11, 14, 21, 28, 35, 42, 49, and 56 post-injury.

**Foot Fault**: The Foot Fault Analysis measured forelimb coordination. Rats were placed on test tube holder grid and allowed to walk. The number of left and right
forelimb faults was counted over 50 steps. To be counted as a fault, the animal’s forelimb must have completely fallen through the grid. A step was counted when both the left and right foot moved consecutively in one direction. Contralateral Faults were calculated.

**Limb Use:** The Limb Use Analysis measured weight bearing use of the forelimbs for exploratory behavior. The animal was placed in a Plexiglas cylinder and videotaped until 10 rears-before-wall were executed. A rear-before-wall occurs when the animal would rear himself upward onto his hind limbs and brace himself with his forelimbs against the Plexiglas. Videos of the animal were rated to examine asymmetries in forelimb use during movements against the wall, during rearing and during landing. Behavioral testing is demonstrated in Figure 2.

**Statistics:** Behavioral data were analyzed with a two-way repeated measures ANOVA followed by post-hoc comparisons of groups at individual time points (Super ANOVA). A p<0.05 indicates a significant difference between groups.

**RESULTS**

**Foot Fault:** Pre-injury testing (Day 0) demonstrated no motor coordination deficits between both forelimbs. Yet, post-injury testing (Day 5) demonstrated an increase in contralateral faults. Within two months post-injury, injections in both cortex and striatum demonstrated a decrease in contralateral foot faults (compared to day 5, post-injury levels). Injecting neurospheres into either the striatum (in Vehicle (M=3.60, SD=3.22) or PBS (M=4.80, SD=3.04)) or cortex (M=4.20, SD=3.32) resulted in significant improvements in motor coordination deficits by day 56 (*p<0.05 compared to CCI Only (M=7.5, SD=3.13) and CCI+PBS only-ST (M=10, SD=4.81)). Interestingly, injecting vehicle alone into the striatum following a CCI (M=4.00, SD=2.82) also resulted in enhanced behavioral function at day 56 (*p<0.05 compared to CCI Only and CCI+PBS only-ST). Injecting vehicle into the cortex (M=5.8, SD=5.13) was not beneficial. Nevertheless, injecting neurospheres suspended in PBS instead of vehicle resulted in behavioral enhancement, suggesting that the behavioral enhancement seen with neurosphere transplantation was not simply a trophic effect of the vehicle. There were no significant differences among groups that received neurospheres into the cortex verses striatum at day 56. Thus, Foot Fault results suggest neurosphere transplantation into either cortex or striatum enhanced recovery and decreases deficits of motor coordination (See Figure 3).

**Limb Use:** Pre-injury testing (Day 0) demonstrated rats used equal percentage of both forelimbs for weight-bearing exploratory behaviors. Following CCI (Day 5), rats preferentially use their ipsilateral (to the injury) forelimb for weight-bearing exploratory behaviors. Transplantation of neurospheres (in either Vehicle (M=46.64, SD=8.09) or PBS (M=51.12, SD=12.01)) into the striatum, but not cortex (M=62.76, SD=15.95) significantly decreased the reliance on the ipsilateral forelimb by day 56 (*p<0.05 compared to CCI Only (M=63.01, SD=13.24) and CCI+PBS only-ST (M=57.22, SD=9.34)). Interestingly, injecting vehicle alone into either the cortex (M=49.63, SD=8.58) or the striatum (M=52.90, SD=12.18) following a CCI also resulted in enhanced behavioral function by day 56 (*p<0.05 compared to CCI Only and CCI+PBS only-ST). Nevertheless, injecting neurospheres suspended in PBS instead of vehicle resulted in behavioral enhancement, suggesting that the behavioral enhancement seen with neurosphere transplantation was not simply a trophic effect of the vehicle. Thus, Limb Use results suggest neurospheres transplanted into striatum, but not cortex, promote recovery of symmetrical limb use (See Figure 4).

**DISCUSSION**

The overall trend throughout our analysis suggests that transplantation of neurospheres post-CCI improves recovery of behavioral function. Neurosphere transplantation into the striatum resulted in improvement in both behavioral tasks compared to controls. Neurosphere transplantation into the cortex resulted in improvement in tests of motor coordination but not symmetrical forelimb use. Vehicle injections...
into the striatum and cortex also enhanced behavioral recovery, albeit not to the level seen following neurosphere transplantation into the striatum. Transplanting neurospheres suspended in PBS into the striatum enhanced recovery, suggesting that the behavioral enhancement is not just a trophic function of vehicle. Transplantation into the striatum, compared to cortex, may be a better site for the enhancement of motor function post-CCI.

This study demonstrates an enhancement of behavioral function following adult neurosphere transplantation in the CCI model of TBI. These findings are consistent with literature suggesting that behavioral recovery can be observed as soon as 14 days following BMSC transplantation, and over a six week period (Hayase 2009; Riess 2007). Although a mechanism has not been fully established, the enhanced efficacy of subcortical neurosphere transplantation post-CCI suggests that the secretion of neurotrophic factors by the neurospheres may be a significant factor in promoting recovery. This potential mechanism may also be the same seen by Hayase (2009) because integration of transplanted cells into the neural circuitry by day 14 is not likely, and any behavioral recovery may be attributed to neurotrophic factors. In addition, studies have identified neurotrophic factors from transplanted human mesenchymal stem cells present as early as two days post transplantation (Kim 2010). The presence of such factors may reduce secondary cell death to promote recovery. Our findings suggest that neurospheres derived from adult bone marrow stem cells may have potential clinical utility as a treatment for traumatic brain injury. Future studies are currently examining the survival of these transplants as well as the effects of the transplants on the size of the contusion and on the expression of trophic factors.

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**TABLE 1**

Seven groups of rats were used in this study to determine the effects of injections and locations. Rats were randomly selected for each treatment group.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>n</th>
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<tbody>
<tr>
<td>CCI only</td>
<td>10</td>
</tr>
<tr>
<td>CCI + Neurospheres (NS) in Vehicle* - Cortex</td>
<td>10</td>
</tr>
<tr>
<td>CCI + Vehicle only - Cortex</td>
<td>09</td>
</tr>
<tr>
<td>CCI + NS in Vehicle - Striatum</td>
<td>10</td>
</tr>
<tr>
<td>CCI + NS in PBS** - Striatum</td>
<td>07</td>
</tr>
<tr>
<td>CCI + Vehicle only - Striatum</td>
<td>09</td>
</tr>
<tr>
<td>CCI + PBS only - Striatum</td>
<td>08</td>
</tr>
</tbody>
</table>

*Vehicle is neurobasal medium supplemented with B27, Fibroblast Growth Factor, and Endothelial Growth Factor.

**Phosphate Buffered Saline (PBS)**
Injections were administered in two deposits surrounding the injury in the cortex or in the striatum. Two 2-µl injections were made of 25,000 cells/µl, totaling 100,000 cells per transplant.

Animal behavior tests: Foot Fault (above) measure forelimb coordination and Limb Use (right) measures weight bearing use of the forelimbs for exploratory behavior.
Improvements in behavioral deficits post transplant for Foot Fault Analysis. Contralateral faults presented for each behavioral testing day.
Improvements in behavioral deficits post transplant for Limb Use Analysis. Percent use of non-injured forelimb presented for testing days 0, 5, 11, and 56.
REFERENCES


