Experimental Neurology 305 (2018) 13–25

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Cell based therapy enhances activation of ventral premotor cortex to improve recovery following primary motor cortex injury

Mary E. Orczykowski[a,*,1,2], Kevin R. Arndt[a,1], Lauren E. Palitz[a,3], Brian C. Kramer[d], Monica A. Pessina[a], Adrian L. Oblak[a,4], Seth P. Finklestein[e], Farzad Mortazavi[a], Douglas L. Rosene[a,b], Tara L. Moore[a,c]

[a] Department of Anatomy & Neurobiology, 72 E. Concord Street, L-1004, Boston University School of Medicine, Boston, MA 02118, USA
[b] Yerkes National Primate Research Center, 201 Dowman Drive, Emory University, Atlanta 30322, GA, USA
[c] Department of Neurology, 72 E. Concord Street C3, Boston University School of Medicine, Boston, MA 02118, USA
[d] Janssen Scientific Affairs, LLC 800 Ridgeview Drive, Horsham 19044, PA, USA
[e] Stemetix, Inc, 604 Webster St. Needham, MA 02494, USA

ARTICLE INFO

Keywords:
Reorganization
Cell based therapy
hUTC (Human umbilical tissue derived cells)
Cortical damage
Functional recovery
Macaca Mulatta
Ventral premotor cortex
Subgranular zone

ABSTRACT

Stroke results in enduring damage to the brain which is accompanied by innate neurorestorative processes, such as reorganization of surviving circuits. Nevertheless, patients are often left with permanent residual impairments. Cell based therapy is an emerging therapeutic that may function to enhance the innate neurorestorative capacity of the brain. We previously evaluated human umbilical tissue-derived cells (hUTC) in our non-human primate model of cortical injury limited to the hand area of primary motor cortex. Injection of hUTC 24 h after injury resulted in significantly enhanced recovery of fine motor function compared to vehicle treated controls (Moore et al., 2013). These monkeys also received an injection of Bromodeoxyuridine (BrdU) 8 days after cortical injury to label cells undergoing replication. This was followed by 12 weeks of behavioral testing, which culminated 3 h prior to perfusion in a final behavioral testing session using only the impaired hand. In this session, the neuronal activity initiating hand movements leads to the upregulation of the immediate early gene c-Fos in activated cells. Following perfusion-fixation of the brain, sections were processed using immunohistochemistry to label c-Fos activated cells, pre-synaptic vesicle protein synaptophysin, and BrdU labeled neuroprogenitor cells to investigate the hypothesis that hUTC treatment enhanced behavioral recovery by facilitating reorganization of surviving cortical tissues. Quantitative analysis revealed that c-Fos activated cells were significantly increased in the ipsi- and contra-lesional ventral premotor but not the dorsal premotor cortices in the hUTC treated monkeys compared to placebo controls. Furthermore, the increase in c-Fos activated cells in the ipsi- and contra-lesional ventral premotor cortex correlated with a decrease in recovery time and improved grasp topography. Interestingly, there was no difference between treatment groups in the number of synaptophysin positive puncta in either ipsi- or contra-lesional ventral or dorsal premotor cortices. Nor was there a significant difference in the density of BrdU labeled cells in the subgranular zone of the hippocampus or the subventricular zone of the lateral ventricle. These findings support the hypothesis that hUTC treatment enhances the capacity of the brain to reorganize after cortical injury and that bilateral plasticity in ventral premotor cortex is a critical locus for this recovery of function. This reorganization may be accomplished through enhanced activation of pre-existing circuits within ventral premotor, but it could also reflect ventral premotor projections to the brainstem or spinal cord.

* Corresponding author at: Department of Anatomy & Neurobiology, Boston University School of Medicine, 700 Albany Street, W-701, Boston, MA 02118, USA.
E-mail address: meorczyk@bu.edu (M.E. Orczykowski).
[1] These authors contributed equally to this work.
[2] Present Address: Division of Anatomical Sciences, University of Michigan Medical School, Ann Arbor, MI 48104, United States.
[3] Present Address: University of New England College of Osteopathic Medicine, Biddeford, ME 04005, United States.
[4] Present Address: Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202-5120, United States.

https://doi.org/10.1016/j.expneurol.2018.03.010
Received 1 June 2017; Received in revised form 18 February 2018; Accepted 9 March 2018
Available online 11 March 2018
0014-4886/

1. Introduction

Stroke is the leading cause of long-term disability in the United States and approximately 795,000 Americans experience a new or recurring stroke each year. The only current FDA-approved therapy for ischemic stroke is intravenous administration of tissue plasminogen activator (tPA) to return blood flow to the blocked artery, but this is only effective if administered within hours following onset of stroke (Ebinger et al., 2015; Wang et al., 2004). Many patients are unable to receive the tPA treatment due to the narrow therapeutic window, or other contraindications (Fugate and Rabinstein, 2015) and even those who do receive tPA often are left with varying amounts of irreversible brain damage and significant residual impairment. Accordingly, neurorestorative treatments are needed to enhance neuroplasticity and facilitate recovery of function following stroke or other traumatic events.

In both animal models and human stroke patients with motor impairments, physical therapy involving rehabilitative motor tasks can lead to significant improvement in motor function over a period of months following stroke (Calautti and Baron, 2003; Frost et al., 2003). Nevertheless, in humans, complete recovery to pre-stroke function is rare (Gowland, 1987). The central nervous system (CNS) has a limited ability to repair or regenerate neurons due to inhibitory factors released by CNS parenchyma and glial scarring (Yiu and He, 2006). As a result, functional recovery following CNS damage is not a result of neuronal regeneration, but more likely due to a variety of neuroplasticity mechanisms such as axonal sprouting, synaptic reorganization, and changes in myelination (Armstrong et al., 2016; Fields, 2015; Mensch et al., 2015; Pascual-Leone et al., 2012). In fact, there is evidence for cortical neuroplasticity and reorganization following CNS lesions in both humans and animal models (Kaas, 1991; Pons et al., 1988; Seitz et al., 1995). Specifically, there is evidence of increasing cortical reorganization of undamaged motor areas including premotor cortices (Frost et al., 2003; Nudo, 2007; Nudo and McNeal, 2013) and evidence of increased proliferation of neural progenitor cells (Anderson, 2001; Arvidsson et al., 2002; Jin et al., 2006; Li et al., 2002; Lindvall and Kokaia, 2015; Marlier et al., 2015; Minger et al., 2007; Zhang et al., 2013; Zhang et al., 2011). Hence, targeting plasticity and reorganization mechanisms with new therapeutic agents may be one way to enhance more complete functional recovery after cortical injury.

Cell based therapies are an attractive avenue of neurorestorative treatments that have shown largely promising results in preclinical models of stroke. While the mechanism remains unclear, studies suggest that cell based therapies enhance endogenous repair mechanisms through increasing brain plasticity and synaptic remodeling (Savitz et al., 2014). While such neurorestorative treatments for stroke have been assessed preclinically, none have successfully translated to human patients. The Stroke Therapy Academic Industry Roundtable (STAIR) (Fisher et al., 2009) and Stem Cells as an Emerging Paradigm in Stroke (STEPS) (Savitz et al., 2011) committees assessed ways to enhance translation between preclinical and clinical studies. Both the STAIR (Fisher et al., 2009) and STEPS (Savitz et al., 2011) reports recommended the use of non-human primate models to validate and further assess efficacy and safety of promising therapies including cell based approaches. Further, STEPS recommends performing appropriate histological studies to examine the effects of the cell based therapy on the remodeling of surviving structures (Savitz et al., 2011).

To that end, this report is a histological follow-up to our earlier study that a cell based therapy of human umbilical tissue derived cells (hUTC) enhanced recovery of fine motor function in our reproducible non-human primate model of cortical injury (Moore et al., 2013). Specifically, it was demonstrated that intravenous administration of hUTC, 24 h after cortical damage, significantly improved function and strength of the impaired hand in the first two weeks of recovery and improved finger-thumb grasp rating during the 12-week post-operative assessment as compared to placebo treated controls.

We hypothesized that the recovery of function observed with hUTC treatment may be due to reorganization of undamaged motor areas and increased proliferation of neural progenitor cells. To evaluate this hypothesis, we report here quantitative analysis in bilateral premotor cortices of c-Fos as a marker of cell activation and synaptophysin as a marker of synaptic density. We also report quantitative analysis of BrdU positive neural progenitor cells in the subventricular zone and subgranular zone as a marker for cell proliferation.

2. Materials and methods

2.1. Subjects

Eight adult male rhesus monkeys (*Macaca mulatta*), ranging in age from 8.5 to 12.1 years, were used in this study. All were part of our previous study (Moore et al., 2013) that assessed the efficacy of hUTC therapy on recovery of motor function following cortical injury. Prior to entering the previous study, all monkeys received medical examinations and were screened to ensure that they did not have a history of malnutrition, diabetes, chronic illness, or any neurological diseases. All monkeys were given initial pre-operative MRI scans to ensure no occult brain abnormality. While enrolled in the study, the monkeys were housed in the Laboratory Animal Science Center of Boston University Medical Campus, which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institute of Health's Office of Laboratory Animal Welfare and were approved by the Institutional Animal Care and Use Committee (IACUC) of the Boston University Medical Campus.

2.2. Fine motor testing and lesion of the M1 hand area

All motor testing and surgical procedures were completed as part of the previous study (Moore et al., 2013) and have been previously described (Moore et al., 2010, 2012, 2013). In the following, the testing and surgical procedures are described briefly. Monkeys were trained on a fine motor task, modified version of a Klüver board (Klüver, 1935), to reach asymptotic performance and the preferred or dominant hand was determined using free choice trials in the testing apparatus. The lesion was then targeted to the hemisphere controlling the dominant hand to ensure that monkeys would be motivated to use the impaired hand during post-operative testing. All subjects then underwent an electrophysiologically guided lesion limited to the hand representation of primary motor cortex. Following exposure of the cortex, the lesion was created by inserting a small glass suction pipette under the pia and bluntly dissecting the small penetrating arterioles as they enter the underlying cortex, producing an ischemic lesion of the gray matter with preservation of underlying white matter (Moore et al., 2013). Twenty-four hours after the lesion, monkeys were given hUTC (CNTO 0007; Advanced Technologies and Regenerative Medicine, LLC – Johnson & Johnson, New Brunswick, NJ) or placebo via intravenous infusion at a dose of 10 million cells/kg and a rate of 0.5 mL per minute. Two weeks following surgery, monkeys were retested on the same fine motor task for 12 weeks with 70% of trials to the impaired hand and 30% to the unimpaired hand. Outcome measures included recovery time, the number of days to return to pre-operative performance, and grasp assessment, determined by a licensed Occupational Therapist (M.A.P.) using our Grasp Assessment Scale for non-human primates (NHP). The scale was adapted from scales used in human stroke patients (Carr et al., 1985; Fugl-Meyer et al., 1975; Whishaw et al., 2002) and consists of hierarchical categories from 0 (no movement) to 8 (normal grasp with accurate pinch between thumb and finger). All relevant subject information, including treatment and behavioral outcome measures, is summarized in Table 1. For all procedures, cognitive testers, surgeons and other research staff were blind to treatment condition throughout