AANS/CNS Joint Cerebrovascular Annual Meeting
Los Angeles, California
February 15-16, 2016

Rodent Model for Enhanced Trans-Arterial Cellular Therapy After Ischemic Stroke by Blood-Brain Barrier Modulation via Sphenopalatine Ganglion Stimulation

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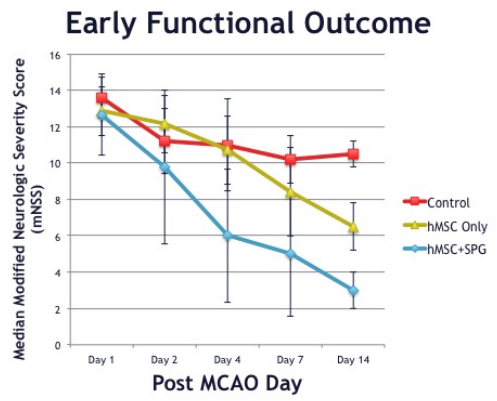
Introduction

The presence of the blood-brain barrier (BBB) in vertebrates is a major limitation to the delivery of therapeutic agents into brain tissue. It has recently been demonstrated stimulation of the sphenopalatine ganglion (SPG) enables rapid and reversible opening of the BBB. We have developed a novel model in rodents to demonstrate the potential utility of SPG stimulation-mediated BBB modulation. The experimental model utilizes SPG stimulation during the administration of mesenchymal stem cells to enhance the passage of administered stem cells, and potentially improve recovery following MCA occlusion.

Methods

Surgical Procedure: Sprague-Dawley rats (n=25) weighing approximately 300g underwent induced ischemia by middle cerebral artery occlusion (MCAO) method. The negative control group (n=5) underwent MCAO alone. Twenty-four hours following MCAO, remaining animals were injected with approximately 1x106 human MSCs into the ipsilateral internal carotid artery while under cyclosporine A immunosuppression. Positive controls (n=10) underwent MSC infusion alone. Immediately prior to MSC infusion, the experimental group (n=10) underwent bipolar stimulation of postganglionic parasympathetic fibers of the SPG within the orbit to selectively open the ipsilateral BBB. Stimulation was delivered at 1ms square-wave pulse width, at 10Hz and 5V continuously for 90s followed by 60s off period. This was continued for 4 cycles, at which time MSCs were delivered by microcatheter within the ipsilateral CCA at 200 µL/min. All animals underwent neurologic testing by modified Neurologic Severity Score (mNSS) on post-stroke days 1,2,4,7, and 14. Immediately prior to MSC infusion, the experimental group (n=10) underwent bipolar stimulation of postganglionic parasympathetic fibers of the SPG within the orbit to selectively open the ipsilateral BBB. Stimulation was delivered at 1ms square-wave pulse width, at 10Hz and 5V continuously for 90s followed by 60s off period.

Figure 1.



mNSS for all experimental animals. MSC injection improved neurologic function, and the effect is augmented when hMSCs are administered following SPG stimulation.

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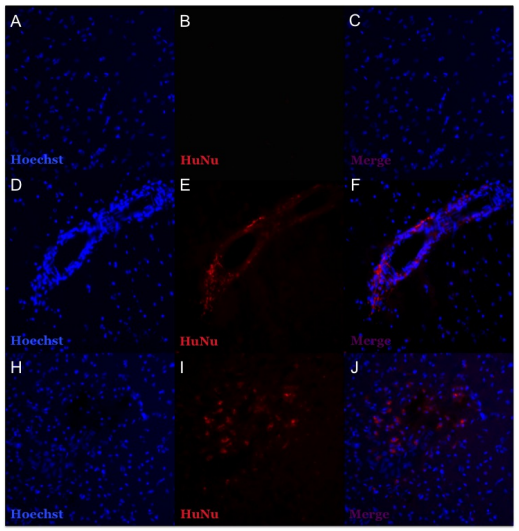
Brains were perfusion fixed and immunofluorescent staining was performed with anti-HuNu and Hoechst 33342.

Results

Functional Outcomes: Modified Neurological Severity Score was measured for all animals (range=0-18) (Figure 1). For control animals, mean scores on post-stroke days 1,2,4,7 and 14 (±std dev) were 13.6±1.1, 11.2±1.8, 11.0±2.5, 10.2±1.3, and 10.5±0.7, respectively. For animals undergoing hMSC injection without SPG stimulation, mean scores were 12.9±1.3, 12.1±1.6, 10.7±1.9, 8.4±2.4, and 6.5±1.3, respectively. For animals undergoing hMSC injection with SPG stimulation, mean mNSS were 12.7±2.2, 9.8±4.2, 6.0±3.7, 5.0±3.4, and 3.0±1.0, respectively. No animal exhibited worsened neurologic status following stem cell injection

Figure 1.



– Immunostaining at 20x magnification demonstrates the effect of intervention on the ipsilateral hemisphere following MCAO in control (a-c), unstimulated (d-f), and stimulation-enhanced (h-j) groups. Anti-HuNu staining demonstrates the presence of human MSCs restricted to the intra/peri-vascular space in unstimulated animals, but clear engraftment into the background neuropil in those undergoing SPG-stimulation.

Histologic Outcomes:
Control animals demonstrated no evidence of anti-HuNu staining. In animals undergoing hMSC injection without SPG stimulation, labeled cells were identified in the perivascular space without significant extension into the surrounding parenchyma within the ischemic penumbra. In animals undergoing arterial injection of hMSCs with SPG stimulation, there was significant evidence of stem cell engraftment into surrounding parenchyma in the ischemic penumbra and normal cortex.

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Conclusions

Early results suggest SPG stimulation-mediated BBB modulation may enhance the graft yield of trans-arterial cellular therapy. The future implications of SPG stimulation for blood-brain barrier modulation in the setting of cellular therapy will also be discussed.

Learning Objectives

Discuss BBB modulation as a potential modality for enhanced trans -arterial delivery by sphenopalatine ganglion stimulation.

Acknowledgements
Funding for this work was supported by grants from the Joesph and Marie Field Family Foundation and the Mary E. Groff Charitable