Rescue of neocortical circuit deficits with modified bone marrow-derived mesenchymal stem cells, SB623, in a rat model of photothrombotic stroke

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Introduction

Cortical injuries, such as those caused by stroke are a major cause of long-term disability[1]. Currently there are no pharmacological therapies on the market that are able to treat chronic sequelae from stroke. This chronic damage can leave individuals with paraplegia that persists indefinitely after the initial injury.

Recently, various types of stem cells have arisen as a potential treatment for chronic damage following stroke in human patients.

SanBio Inc. recently completed a Phase 1/2a clinical trial with intracranial implantation of modified bone marrow-derived mesenchymal stem cells, SB623, which appear to be safe and were associated with improvements in clinical outcome in some patients for 24 months post implantation. However, the mechanisms by which these cells reduce neurological deficits remain unknown. Past research indicates that cortical stroke can lead to hyperexcitability in surrounding circuits and increased inflammation. Therefore in this preclinical study, we study whether SB623 can ameliorate circuit deficits through slice electrophysiology, and decrease inflammation by staining for GFAP and Iba1.

[1]Emelia J. Benjamin et al. "Heart Disease and Stroke Statistics 2017: A Report From the American Heart Association." *AHA Journal.* Volume 136, Issue 17. 2017.

Methods

Photothrombotic Stroke Model

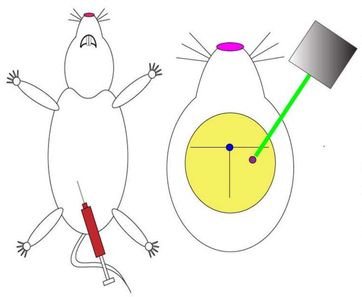


Fig. 1 IP Injection of **Rose Bengal** Solution (40 mg sol/kg rat), which is a light sensitive dye that creates free radicals in the artery after exposure to light, which in turn induce stroke. Placed Light source (562 nm) on **S1 cortex** at the coordinates (AP -2.5 , ML +5.0).

SB623 & Vehicle Implantation

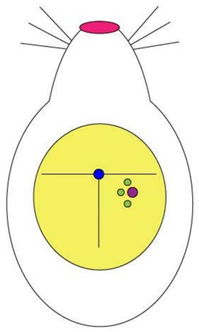


Fig. 2 Implanted SB623 or vehicle into **periinfarct region** of the S1 Cortex 28 days after induction of stroke. Three injection sites, located at (AP -1, ML +4, DV 1.8), (AP -2.5, ML +3.5, DV 1.4), (AP -4, ML +5, DV 1.3), were used. The same implantation protocol was used for the electrophysiology, cell survival and immunohistochemistry studies.

Methods

LFP Slice Recordings

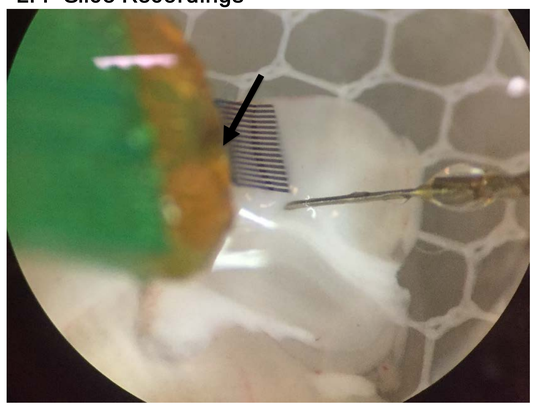


Fig. 3 . Using slice multi-array electrophysiology we measured the changes in the cortical circuitry by recording the local field potential (LFP) across the different cortical layers and assessing the current source density. 16-Channel Neuronexus probes were placed in the cortical column, 1 mm from the stroke region, and stimulated by a probe placed directly below in the white tract. Stroke area is under the green recording probe, indicated by the black arrow. 14 days after implantation of SB623 or vehicle (plasmalyte), animals were sacrificed and their brains were sliced on a Leica vibrotome in 400um sections. Animal tissue was kept alive and suspended in oxygenated artificial cerebrospinal fluid (ACSF) for the duration of the recordings.

Results

1. Cell survival

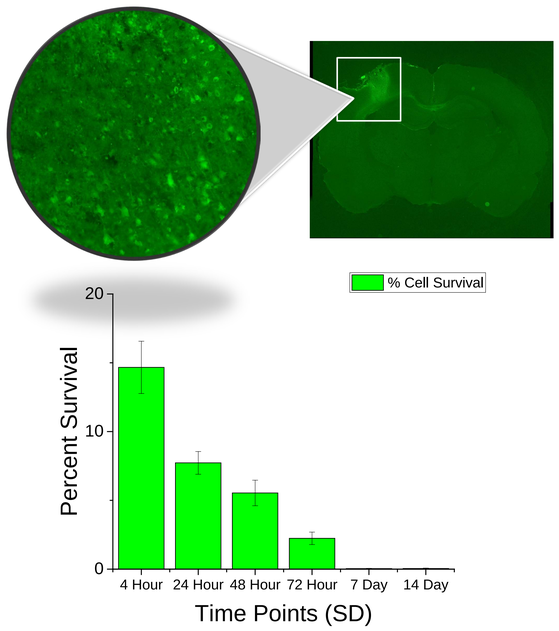
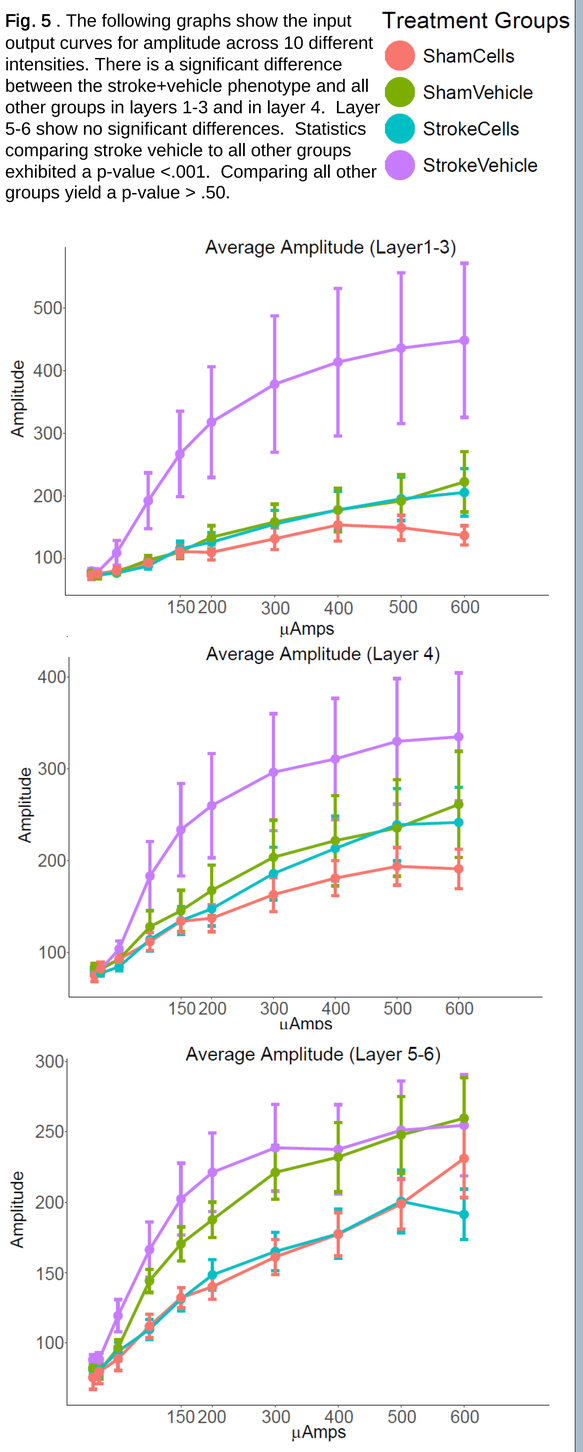


Fig. 4 Square box is highlighting the stroke area and the presence of SB623. Green fluorescent specks are the presence of the injected SB623 by staining for Stem 121, a human cell marker. After staining, the slices were imaged and cells were counted using image J. Percentage cell survival was then calculated.

Results

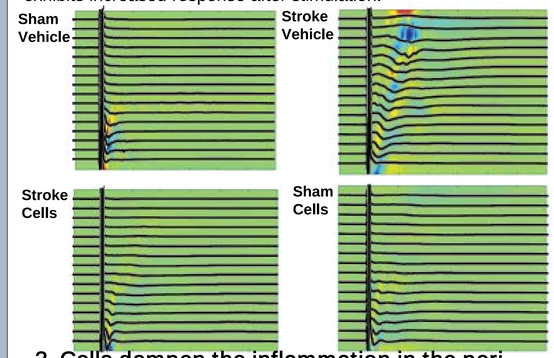
2. Cells dampen the cortical network hyperexcitability after stroke

Fig. 5 . The following graphs show the input output curves for amplitude across 10 different intensities. There is a significant difference between the stroke+vehicle phenotype and all other groups in layers 1-3 and in layer 4. Layer 5-6 show no significant differences. Statistics comparing stroke vehicle to all other groups exhibited a p-value <.001. Comparing all other groups yield a p-value > .50.



Results

Fig. 6 . Below are CSDs and traces for each of the groups. The Y-axis shows responses from the 16 channels (1-16 up). The X-axis shows the time interval of 20 seconds post stimulation. Stroke Vehicle group exhibits increased response after stimulation.



3. Cells dampen the inflammation in the peri-stroke cortex

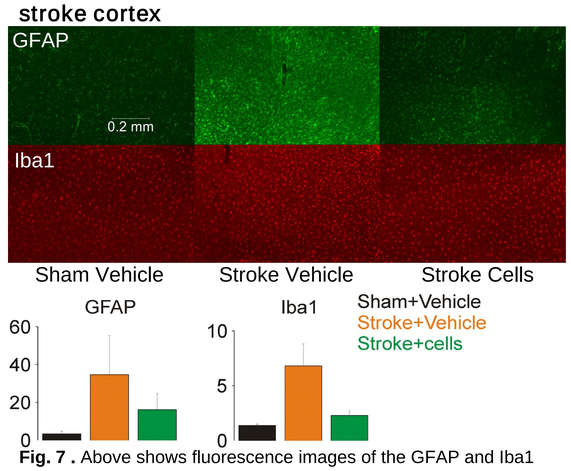


Fig. 7 . Above shows fluorescence images of the GFAP and Iba1 stains in the cortex lateral to stroke region. At right is quantification of the above images, comparing ipsilateral to contralateral fluorescence. Quantification suggests that SB623 ameliorates inflammation in the peri-stroke region.

Conclusions

From the results obtained in the previous studies we can conclude the following:

- After injection of SB623 in the peri-stroke region, cells persist through 72 hours but are completely gone by the 7 day time point.
- Electrophysiological results confirm that stroke injury leads to hyperexcitablity in the peri-stroke region as seen in amplitude (shown left) and line length (not shown) in the superficial layers.
- Electrophysiological results also show that treatment with SB623 dampens the cortical network hyperexcitability back to normal levels.
- Immunohistochemistry results suggest that cortical hyperexcitabiltiy results from increased inflammatory activity in the peri-stroke region, which is subsequently dampened by introduction of SB623 back to close to normal levels.

Acknowledgements

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