Wnt/ beta-catenin Signaling Is Active Post-stroke and Wnt-3a Liposomes Promote Neurogenesis and Functional Recovery Post-MCAO

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Introduction

Wnt/ beta-catenin signaling has been identified as an essential component of adult neurogenesis within the hippocampus. Its role within the adult Subventricular zone, SVZ, is less well understood, as is its role in the context of post-stroke neurogenesis. With this work we aim to 1) determine the presence of active Wnt/ beta-catenin signaling following experimental focal cerebral ischemia 2) identify the cell types that it is upregulated by 3) establish its effects on neurogenesis, regeneration and recovery following stroke through administering a liposomal Wnt-3a protein preparation directly into the brain parenchyma.

Methods

- Axin 2 +/- male mice, reporter for Wnt/ beta-catenin signaling, were subjected to 30 min Middle Cerebral Artery Occlusion, MCAO. Activation of the pathway was determined via immunohistochemistry against beta-galactosidase.

- C57/ Bl6 male mice were subjected to 30 min of MCAO. Wnt-3a liposomes, made and *in vitro* tested in the lab, were injected intraparenchymally at 2, 4 and 6 days post-MCAO. Behavior tests: the horizontal ladder test was performed at 2 days prior to and at 1, 2, 3 and 4 weeks post stroke. Mice were sacrificed at 1 month post MCAO.

Results

Results (1)

- Wnt/ beta-catenin signaling is upregulated at 1 and 3 days post MCAO but is unchanged compared to SHAM operated animals at 5 and 7 days poststroke (Figure 1)

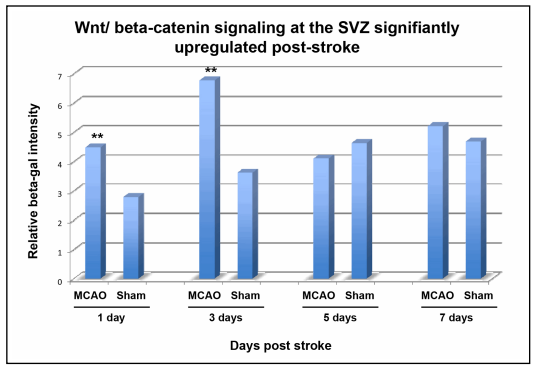

Figure 1. Wnt/ beta-catenin signaling is upregulated at the neurogenic Subventricular Zone, SVZ, at 1 and 3 days post MCAO, but it is unchanged compared to SHAM operated animals at 5 and 7 days post-stroke. Intensity measurments of beta-galactosidase were obtained using fluorescent microscopy under uniform conditions and intensity levels between MCAO and SHAM operated animals compared using ImageJ.

Results (2)

- the main site of Wnt/ beta-catenin pathway upregulation is the Subventricular zone, SVZ, a site of endogenous neurogenesis, however, it is also active within certain cells in the penumbra, as well as, in the healthy cortex and striatum (Figure 2)

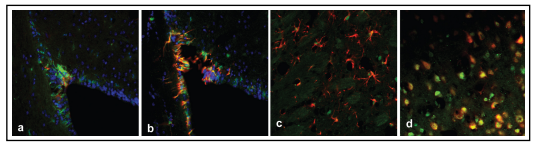

Figure 2. Wnt/ beta-catenin positive cells (green) colocalize with Nestin (a) and Doublecortin, DCX, (b) expressing neuronal progenitors at the adult Subventricular Zone, SVZ, 3 days post-stroke (in red). Mature neurons, positive for NeuN (d), and GFAP expressing reactive astrocytes and neural progenitors at the infarct border (c), were also positive for the Wnt/ beta-catenin pathway . Colocalization was determined at 3 days post-MCAO.

Results (3)

- intraparenchymal injection of Wnt3a liposomes, prepared in the lab, robustly activates the pathway both *in vitro* and *in vivo* and enhances endogenous neurogenesis (p<0.001, t-test), evident at 1 month post-MCAO (Figure 3)

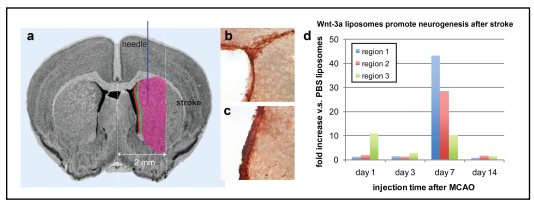

Figure 3. Intra-parenchymal Wnt-3a liposomal injections generated consistently higher numbers of Doublecortin, DCX, positive neural progenitor cells both at the SVZ (b, c) and within the striatum, when compared to PBS liposomal injections (d). This augmentation was greatest when Wnt -3a was administered 7 days after ischemia, with most DCX cells present within SVZ proper (red in a) and 100 um from SVZ proper (green in a), the latter comprising migrating cells.

Results (4)

-functional recovery was significantly improved following repetitive Wnt-3a liposomal administration post-stroke, as determined by the horizontal ladder behavior test at 4 weeks post-MCAO (p<0.010, t-test, **). There was also a trend of smaller infarct sizes in the Wnt-3a injected cohort (data not shown)

Conclusions

We have determined that the Wnt/ beta-catenin pathway may indeed play an important role in adult post-stroke neurogenesis and functional recovery. The pathway is upregulated early after stroke within the neurogenic SVZ, and it is also present within reactive astrocytes and mature neurons. Exogenous activation of the pathway post-stroke through a novel liposomal preparation significantly enhances endogenous neurogenesis and promotes functional recovery in the long term.

References

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