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DISSERTATION

**“EFFECTS OF TGFβ RECEPTOR INHIBITION TO PREVENT CELLULAR DEATH AND PROMOTE RECOVERY IN NEONATAL HYPOXIC ISCHEMIC BRAIN INJURY”**

by

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**ABSTRACT**

Cerebral hypoxia-ischemia (HI) is the predominant contributor to neonatal encephalopathy, the most important cause of neurological morbidity in infants. This insult triggers a cascade of neuroinflammation lasting several days that causes progressive damage to the infant brain. The cellular processes mediating this prolonged neurodegeneration in HI injury are not fully understood, impeding the development of therapeutics that can reduce brain damage. Consequently, there are no therapies that are fully protective and none that specifically target mediators of chronic inflammation and injury. We previously established the cytokine transforming growth factor (TGF)β1 to be highly elevated following HI, and delivery of an antagonist for TGFβ receptor activin-like kinase 5 (ALK5), in a rat model of moderate perinatal HI produced significant improvement in neurological outcomes. This ALK5 antagonist, SB505124, was administered systemically by osmotic pump beginning 3 days following HI. SB505124-treated animals injured on postnatal day 6 (P6) sustained less damage to their hippocampi and had improved performance on the Morris water maze (MWM) when tested on P60 versus vehicle-treated HI animals. By contrast, SB505124 did not improve sensorimotor deficits and exacerbated hippocampal and thalamic volume loss when administered 3 days after HI to P9 pups. SB505124-treated rats injured on P9 tended to perform worse than their vehicle- treated counterparts on MWM, and SB505124 treatment did not preserve hippocampal or thalamic neurons in P9 pups when combined with therapeutic hypothermia. Our results demonstrate that there is a dynamic switch in the CNS response to TGFβ1 that occurs around P9 in rats in which TGFβ signaling inhibition worsens functional outcomes. This response is similar to the outcome of antagonizing TGFβ signaling in adult stroke and other CNS disease models. In the P6 HI model, SB505124 yielded a ~90% reduction in actively apoptotic cells one week after injury. To elucidate the mechanism whereby ALK5 inhibition reduced neuronal death in the P6 HI model, we assessed levels of autophagy markers in neurons of the neo-cortex, hippocampus, and thalamus, and in the subcortical white matter, and found that SB505124 increased numbers of autophagosomes and levels of lipidated LC3 (light chain 3), a key protein known to mediate autophagy. Ex vivo studies to assess for autophagic flux using the lysosomal inhibitor chloroquine confirmed an enhancement of autophagy with SB505124 in the injured hemisphere, with a significant buildup of autophagic proteins LC3 and p62 in SB505124 + chloroquine treated brain slices. To determine if enhanced autophagy is directly responsible for improved outcomes, we independently activated autophagy using stimulatory peptide Tat-Beclin1. Induction of autophagy via Tat-Beclin1 starting 3 days after injury preserved the hippocampus and thalamus and led to improved sensorimotor function. We conclude that SB505124 is an effective treatment for HI-related encephalopathy in moderately preterm infants, offering protection of the neocortex, hippocampus and thalamus via enhanced cerebral autophagy contributing to decreased progressive cell death. Stimulating autophagy alone in the subacute stage post-injury may also be a therapeutic strategy, providing modest benefits in neurobehavioral function.