Applying pre-clinical methodologies to better understand the genetic and physiologic mechanisms underlying brain injury: Melatonin as an exemplar

Nicole Osier,1,2 Sheila Alexander,1,3 C. Edward Dixon2,3

1. University of Pittsburgh, School of Nursing, Department of Asia & Eastern Care, Pittsburgh, PA, USA
2. University of Pittsburgh, School of Medicine, Department of Neurosurgery, Pittsburgh, PA, USA
3. University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA

Purpose: To gain proficiency in basic and bench science methods via completing a dissertation study characterizing the melatoninergic

Methods, Results, and Discussion

Injury and Factors Controlled For

Injury

- CCI (n = 10) and Sham (n = 10)
- All animals were treated with identical techniques sans impact
- Anesthesia: 7 mg/kg ketamine (i.p.); 3 mg/kg xylazine (i.p.)
- Control over potential confounders: age, sex, weight, diet, genotype, etc.
- Randomization of animals prior to injury

Controlled Factors

- Depth of anesthesia
- Age: Young adult (≤ 25 weeks old at time of surgery) or middle-aged (≥ 125 weeks old)
- Sex: Male
- Mice: BALB/cJ (Harlan Laboratories Inc.)
- Rats: Sprague-Dawley (Charles River Laboratories Inc.)
-Treatment of Animals

Treatment of Animals

- CCI-induced: animals were treated with identical techniques sans impact
- Sham: no impact with anesthesia and craniectomy
- Treatment: 10 min of ischemia
- Sacrifice time point: 24 hr post-surgery

Data Quantification and Analysis

Data quantification

- Behavioral outcomes: used in a craniectomy and sham setting
- Semi-dry transfer
- Gel running conditions: 165 Volts for approximately 30 minutes
- Western blot
- Compared to 8 protein standards to determine μg protein per μL sample
- 2 μL of protein per well (diluted 5 fold with 8 μL of deionized water)
- Stripped with Restore Stripping Buffer and washed between 1' antibodies

Wet Laboratory Methods

- Washed in TBS-T, blocked in 5% milk, incubated in 1% antibody overnight
- Confirmed gel ran without error
- Pooled hippocampal samples (p=0.04)
- Hippocampal tissue (p= 0.011)
- Cortical tissue (p= 0.002)
- 136F3
- 136F3

Wet Laboratory Methods

- MT2 in Hippocampus

Melatonin (MEL)

- Melatonin, a 17-atom radical, is distributed throughout the CNS
- Role in TBI remains unknown
- MT1 and MT2 found in the brain
- MEL-specific receptors
- G-protein receptor dependent mechanism poorly understood
- Anti-oxidant effects extensively studied
- May be involved in response to TBI but insufficient for full neuroprotection

Background

- TBI symptom profile is well-characterized
- Extensive research into pathophysiology of 1st and 2nd injury

State of the science for TBI

- Everyone is at risk (all ages; sexes; etc.); some at elevated risk
- No new FDA-approved therapies
- Successful phase II trials (hypothermia, progesterone, cyclosporine)

Conclusions and Future Directions

- Avenues for clinical exploration if warranted
- Association with MEL levels and other outcomes
- Determination if the effects of MEL therapy are receptor-dependent
- Few studies showed adverse effects (Cirak, 1999; Jadhav; 2000)
- Many promising effects (Ates, 2006; Babaee, 2015; Campolo, 2013; many others)
- MT1 and MT2 downregulation may affect response to therapy
- Behavioral outcomes
- Downstream effects of GPCR

Current challenges

- Role in TBI remains unknown
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- The International Society for Nurses in Genetics
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Future Directions

- MT1 in Hippocampus

- Pharmacologic, a role in MT1 and MT2 in TBI is supported
- MT2 is downregulated in response to TBI
- MT1 is downregulated in response to TBI
- Our future work uses KO: CCI (n = 3)
- Sham (n = 3)
- CCI
- Sham

- MT1 in Hippocampus

- Still, model is well-validated
- Complicate interpretation
- Not tested in mice
- HDN polymorphisms exist
- Huntington’s disease
- MT1 is a MEL receptor classified as a G-protein coupled receptor (GPCR)
- First study to report downregulation of MT1 after TBI (in rats and mice)
- First study to report downregulation of MT2 after TBI (in rats)

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