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Characterization of ABIN-1 in the Traumatically-Injured Brain

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School of Medicine

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INTRODUCTION

Traumatic brain injury (TBI) is associated with chronic pain and persistent neuroinflammation. Opioids are often prescribed in order to relieve pain symptoms, but recent evidence suggests that their use negatively impacts the neuropathology of TBI, contributing to exacerbation of behavioral impairments and neuroinflammation. To understand the mechanisms that may underlie these outcomes, the following signaling pathways were investigated in the present work:

- The de-ubiquinating enzyme tumor necrosis factor α-induced protein (TNFAIP)3 or A20, inhibits the inflammatory signaling transcription factor nuclear factor (NF)-κB leading to attenuation of the inflammatory response
- A20-binding inhibitor of nuclear factor κB (**ABIN-1**), which physically interacts with A20, also plays a role in the inhibition of NF-κB

Recent evidence demonstrated by Zhou, et al[1] suggests that ABIN-1 negatively regulates μ-opioid receptors. Therefore, dysregulation of ABIN-1 may contribute to the neuropathology post-TBI, especially after treatment with opioids.

METHODS

Phase 1: Determine the protein expression of ABIN-1 in various brain regions and the influence of neuroinflammation on ABIN-1

Previously, it was determined that ABIN-1 regulates the lipopolysaccharide-(LPS) induced inflammatory response in chondrocytes[2]. Therefore, to determine the role of neuroinflammation in ABIN-1 protein expression, 10-week-old male mice were injected intraperitoneally with a single dose (10 mg/kg) of LPS or saline (control). Brain tissue and various peripheral tissues were harvested and flash frozen 24 hours after LPS injection.

Phase 2: Determine the effect of TBI on ABIN-1 protein expression over time

The effects of TBI on ABIN-1 protein expression will be investigated using brain tissue from mice subjected to a moderate level TBI after 1, 7, and 10 days.

Phase 3: Determine the effect of morphine treatment post-TBI on ABIN-1 protein expression

Zhou et al. demonstrated that ABIN-1 negatively regulates μ-opioid receptors in an ABIN-1 overexpression model[1]. Considering this evidence, morphine exposure post-TBI is likely to influence ABIN-1 expression. Phase 3 will investigate this influence in brain tissue harvested from mice treated with morphine after a moderate level TBI.

Analysis of tissue in each phase involved or will involve the following:

- Immunoblot
- Co-immunoprecipitation assays
- RT-PCR

Antibodies: Cell Signaling – Anti-ABIN-1 polyclonal antibody #4664

RESULTS

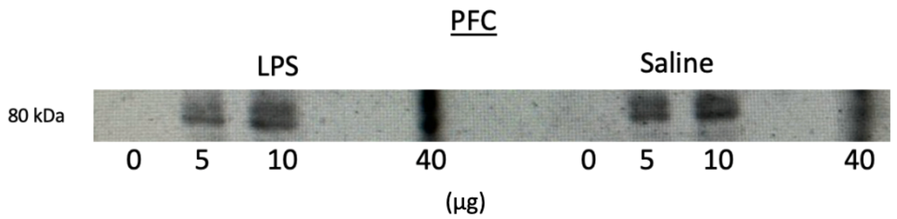

Figure 1: Fluorescent immunoblot exhibiting a concentration gradient (μg) of ABIN-1 expression at 80 kDa in prefrontal cortex (PFC) tissue harvested 24 hours after treatment with lipopolysaccharide (LPS) or saline.

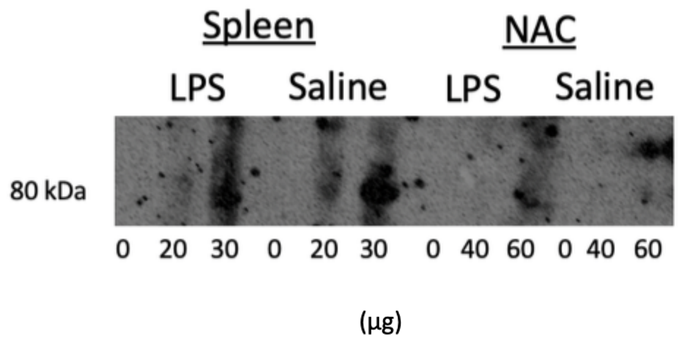

Figure 2: Enhanced chemiluminescence (ECL) immunoblot exhibiting a concentration gradient (μg) of ABIN-1 expression at 80 kDa in the nucleus accumbens (NAc) harvested 24 hours after treatment with lipopolysaccharide (LPS) or saline and splenic tissue harvested 2 hours after treatment with LPS or saline.

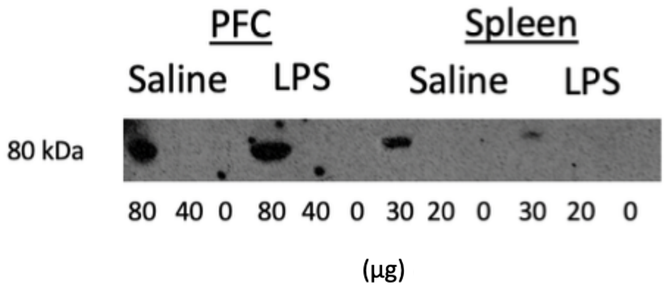

Figure 3: Enhanced chemiluminescence (ECL) immunoblot exhibiting a concentration gradient (μg) of ABIN-1 expression at 80 kDa in the prefrontal cortex (PFC) harvested 24 hours after treatment with lipopolysaccharide (LPS) or saline and splenic tissue harvested 2 hours after treatment with LPS or saline.

DISCUSSION

These results are preliminary. Work is still being completed to investigate ABIN-1 protein expression in various brain regions including the dorsal hippocampus, ventral hippocampus, dorsal striatum, amygdala, anterior cingulate cortex, and thalamus. Figure 1 & 2 demonstrate that LPS may modulate ABIN-1 expression in the prefrontal cortex (PFC) and the nucleus accumbens (NAc), 24 hours after treatment with LPS.

Previous work suggests that ABIN-1 is highly expressed in the splenic tissue[3]. Therefore, splenic tissue harvested from mice 2 hours after treatment with a single dose of LPS was used as a peripheral control of ABIN-1 protein expression. Interestingly, Figure 2 & 3 may demonstrate a reduction in splenic expression of ABIN-1, 2 hours after treatment with LPS. This may be due to the downregulation of ABIN-1 protein expression after stimulation with LPS, which is contradictory to the findings in other investigations[2]. However, this may also be due to the mobilization of white blood cells from the splenic tissue after activation by LPS. If this is the case, we would expect ABIN-1 expression to be reduced since there are less immune system modulating cells present in the splenic tissue. Further work is being completed in order to investigate this observation.

Since these findings are preliminary and the method is still being optimized, it is difficult to make conclusions from the presented data. However, further investigation may elucidate the role of ABIN-1 in the central nervous system. The findings of Zhou et al. have demonstrated a relationship between inflammatory and μ-opioid receptor signaling. As stated previously, TBI has been linked to chronic pain. Opioids are often prescribed in order to relieve symptoms. Since evidence suggests that opioids may exacerbate neuroinflammation in the injured brain, the role of the anti-inflammatory protein, ABIN-1, is of particular interest.

ACKNOWLEDGEMENTS

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