Investigating Mechanistic Pathways Involved in Neuroimmune Dysregulation and Neuropathic Pain Due to Prenatal Alcohol Exposure

Ariana Pritha, Michela Dell’Orco, Joshua J. Sanchez-2, Jacob E. Sanchez-2, Suzy Davies-1, Daniel D. Savage-1, Nikolaos Mellios-1, Shahani Noor-1, Erin. D. Milligan-1

(1) Department of Neurosciences, School of Medicine, University of New Mexico, Albuquerque, NM, 87131, USA.
(2) Department of Neurosciences, School of Medicine, University of California, San Diego, CA, USA

Introduction

Neuropathic pain is a chronic pain condition resulting from damaged peripheral nerves that induces allodynia (pathological touch sensitivity). Our previous studies suggest that prenatal alcohol exposure (PAE) is a risk factor for allodynia. In contrast to control rats, adult PAE rats with minor nerve injury develop allodynia. Nerve-injured PAE rats display heightened peripheral and central nervous system (CNS) immune cell-derived proinflammatory cytokine production that consequently results in allodynia. This suggests that PAE dysregulates peripheral immune and CNS glial cell function to over-represent immune challenges leading to allodynia. However, gene expression profiles underlying dysregulated neuroimmune activity due to PAE are poorly understood. For the current study, the bioinformatics software Ingenuity Pathway Analysis (IPA), was used to gain insight into key pathways and molecular targets underlying PAE effects on dysregulated neuroimmune function. Non-coding circular RNAs (circRNAs) are novel modulators of mRNA expression regulating CNS glial-immune function. Therefore, we hypothesized that circRNAs might be associated with critical neurological and immune dysfunction induced by PAE. Through IPA we identify the canonical pathways, diseases, and molecular functions, associated with the gene networks altered by PAE and nerve injury. Our microarray data identified 114 circRNAs that were differentially expressed in the spinal cord from PAE rats than in control rats following minor nerve injury, with a p-value of <0.05 and with a fold change >1.5. Additionally, our data identified 15 circRNAs in blood leukocytes that displayed significantly different expression patterns as a long-term consequence of PAE in the absence of any injury. Analysis of “top networks” of the genes associated with these differentially expressed circRNAs identified distinct canonical pathways including NF-kB which is activated by innate immune receptor TLR4 signaling. These results suggest that circRNAs dysregulation could be a novel underlying mechanism of PAE-induced neuroimmune dysregulation.

Methods

Prenatal Alcohol Exposure and Chronic Constriction Injury

1. Long Evans rats exposed to saccharin (Sac control) or 5% ethanol (PAE) through voluntary drinking paradigm.
2. Offspring were then either subjected to minor 1-suture chronic constriction injury (CCI) in their unilateral sciatic nerve or a sham surgery (no injury).
3. Von Frey fiber test was used to assess hind paw touch sensitivity of 4-7 month-old female rat offspring.

IPA Core Analysis & Further Top Network Specification

1. Total RNA isolated samples were sent to Agilent Microarray service to conduct the circRNA analysis
2. Core analysis was run on IPA to yield: Global Mechanistic Network, Pathway Analysis, Heat Maps, Canonical Analysis, and associated Mechanisms & Functions.
3. Gene IDs associated with significantly dysregulated circRNAs and the fold change (+ indicating up regulation, - indicating down) was included for IPA analysis.
4. Top networks with a z-score above 20 were included as it indicated a significant relationship. Networks with a z-score > 50 indicate causal relationships.

Results

PAE results in neuropathic pain susceptibility following minor nerve injury

Circular RNA dysregulation and associated gene network analysis from peripheral blood leukocytes at baseline

Figure 2. PAE and Sac control rats were exposed to sham or minor nerve (CCI) injury. With minor nerve injury only PAE rats developed pathological touch sensitivity (allodynia) indicating increased susceptibility of PAE rats to develop allodynia.

Circular RNA dysregulation and associated gene network following nerve injury in the spinal cord

Figure 3A. In the graph above, differentially expressed circular RNAs were determined in blood leukocytes and discriminated based on P<0.05 and |log2 FC| >1.5. There are 3 up-regulated circRNAs and 78 down-regulated. 7% was exonic - 16% sense overlap - 2% antisense - 3% intronic and 4% was intergenic.

Figure 3B. In this dataset, this top network had a score of 37, displaying NF-kB signaling (pink highlight), a key transcription factor of immune cytokines, as an important node. Additionally, following notable aspects were associated with this network; Neuroinflammatory Pain Signaling Pathway, Dorsal Horn Neurons, Glial Signaling Pathway, TLR4 Signaling, Neuroinflammation signaling pathway, and the Glucocorticoid receptor signaling pathway.

Conclusion

• PAE is risk factor for neuroimmune dysfunction and chronic pain
• PAE itself dysregulates circRNAs in the peripheral leukocytes suggesting circular as potential biomarkers
• Dysregulated circRNAs were identified in the spinal cord following nerve injury
• IPA analysis of genes associated with these dysregulated circRNA reveals critical immune-inflammatory pathways including NF-κB signaling
• These bioinformatics analysis provided new insights on potential mechanisms involved in long-term neuroimmune dysregulation due to PAE.

References