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Heath W. Shelton
East Tennessee State University

Russell W. Brown
East Tennessee State University

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Heath W. Shelton[1] and Russell W. Brown[2]

[1] *Department of Biological Sciences, College of Arts and Sciences, East Tennessee State University, Johnson City, TN.*
[2] *Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, Johnson City, TN.*

Abstract

Introduction: Current medical treatment for individuals diagnosed with schizophrenia (SCHZ) primarily relies on the inhibition of the dopamine D2 receptor that has been shown to be supersensitive in these patients. Treatment occurs through the use of antipsychotic medication which leads to a number of debilitating dose-dependent side effects, such as weight gain, agranulocytosis, and seizures. Patients diagnosed with SCHZ have also been shown to have increased inflammation in their central nervous system (CNS), particularly within specific brain regions such as the prefrontal cortex and hippocampus. This is in large part due to the interaction between a pro-inflammatory cytokine called tumor necrosis factor-alpha (TNFα) and microglia, which are resident CNS defense cells. TNFα is a cell-signaling protein, regulates a variety of immune cells, and is involved in the acute phase reaction of inflammation. Upon activation by TNFα secretion, microglial cells switch from being anti-inflammatory (M2) to pro-inflammatory (M1), thereby resulting in neuroinflammation as well as synaptic loss and neuronal death. In this project, we hypothesized oral administration through the diet of a novel TNFα modulator (PD2024) developed by P2D Biosciences, Inc. (Cincinnati, OH) would significantly reduce microglia activation in rats neonatally treated with Polyinosinic:polycytidylic acid (poly I:C). Methods and Results: To test our hypothesis, four groups (Neonatal Poly I:C/TNFα, Neonatal Poly I:C/Control, Neonatal Saline/TNFα, and Neonatal Saline/Control) were intraperitoneally injected with either poly I:C or saline during postnatal days (P)5-7. Poly I:C is an immunostimulant that mimics neonatal infection in humans, which also has been found to be a factor for the development of SCHZ later in life. Between days (P)30-(P)60, the Neonatal Poly I:C/TNFα and Neonatal Saline/TNFα groups were orally administered PD2024 through the diet. After (P)60, brain tissue was evaluated by immunohistochemistry (IHC) and confocal microscopy. Immunohistochemistry was used to label microglial cells in the prefrontal cortex and hippocampus with a green fluorescent dye attached to Iba1, a protein that specifically binds to these cells. Upon completion of IHC, tissue was evaluated using a confocal microscope and then analyzed with NIH ImageJ software. Analysis parameters included cell count, sampled cell body fluorescence, and overall image fluorescence. The results obtained showed a significant decrease in microglia activation for the Poly I:C/TNFα group when compared to the Poly I:C/Control group, as well as similarities in activation levels with the Saline/Control group. These results were demonstrated in both sampled cell body fluorescence and overall image fluorescence measurements. Conclusion: This data supports the hypothesis that PD2024 is successful in reducing microglia activation through the modulation of TNFα. Therefore, treatment with a TNFα modulator such as PD2024 alongside of current antipsychotic medication could mediate neuroinflammation and reduce the dose-dependent side effects. This approach could be a promising therapeutic treatment option for those diagnosed with schizophrenia, as well as potentially for other neurocognitive and behavioral disorders.

Introduction

- Schizophrenia (SCHZ) is a chronic debilitating neurocognitive and behavioral disorder that affects approximately 21 million people worldwide [1].
- Current medical treatment relies on the modulation of the dopamine D2 receptor, shown to have supersensitivity in those diagnosed with SCHZ [2-4].
- Treatment to modulate this receptor through the use of typical and atypical antipsychotic medications has a number of debilitating dose-dependent side effects, including insulin resistance/diabetes, agranulocytosis, and seizures [5].
- Increasing evidence suggests neuroinflammation in humans plays a significant pathophysiological role in SCHZ [6-7].
- Individuals diagnosed with SCHZ have higher levels of inflammation in their CNS, particularly within specific brain regions, such as the prefrontal cortex (pFC) and hippocampus (Hip) [8].
- Rats injected with polyinosinic:polycytidylic acid (poly I:C) between postnatal days 5-7 show increased neuroinflammation during lifespan, consistent with SCHZ [9].
- This study investigates the use of a novel anti-inflammatory compound produced by our collaborators at P2D Bioscience, Inc. (Cincinnati, OH) to reduce neuroinflammation via microglial cell quantification in rats neonatally treated with poly I:C.

Purpose & Hypotheses

The central aim of this study was to determine if treatment with an anti-inflammatory compound (PD2024 – TNFα modulator) can reduce microglia activation (neuroinflammation) in rats neonatally treated with poly I:C.

Hypotheses:

1. **Neonatal poly I:C will result in increased microglial activation.**
2. **Neuroinflammation in the pFC and Hip as a result of neonatal poly I:C treatment will be attenuated by oral administration of PD2024.**
3. **Reduction in neuroinflammatory levels will be similar to control levels.**

Methods

Neonatal Poly I:C Treatment (P5-7)

- Animals were intraperitoneally (IP) administered either poly I:C (2 mg/kg) or saline during postnatal days 5-7.

Dietary Treatment

- All animals were given food ad libitum.
- Animals in the Neonatal Poly I:C/TNFα and Neonatal Saline/TNFα were given a chow that contained PD2024 between P30-P60.
- Animals in the remaining two groups (Poly IC Control and Saline Control) were given a chow that did not contain the drug.
- The two chows were identical up to P30 and did not contain any additional additives.

Immunohistochemistry (IHC)

- Formalin fixed, cryopreserved brain tissues were coronally sectioned on a Leica cryostat at 50 µm thickness and stored at -20ºC until immunolabeling.
- Iba1 (Wako Chemicals USA, Richmond, VA) was the primary antibody used for IHC labeling of microglial cells and AlexaFluor488 conjugated Anti-Rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was the secondary antibody tagged with GFP to emit fluorescence.
- Free floating sections were mounted on glass slides and coverslipped using mounting media.
- Slides were examined under a Leica TCS SP8 inverted confocal microscope at a magnification of 40x.
- 4 images were captured per brain region (4 pFC, 2 CA1 & 2 CA3 of the Hip)

Image Analysis and Quantification

- NIH ImageJ software was used to quantify images of the prefrontal cortex and CA1 and CA3 regions of the dorsal hippocampus.
- Measurements and quantification included manual cell count, average sampled cell body fluorescence, and overall field fluorescence.
- Cell count per given field was measured using two different experimenters who were blind to all conditions.
- The freehand tool in ImageJ was used to draw around a given microglia cell body.
- Integrated Density from ImageJ was used to determine average cell body fluorescence and overall field fluorescence.
- At random, five GFP-stained microglia cells were selected per given field for the measurement of average cell body fluorescence.

Statistical Analysis

- A separate one-way ANOVA was used to analyze each brain area.
- The Newman-Keuls post hoc test (p=0.05) was used to analyze significant interactions (p=0.05).

Figure 1. Prefrontal Cortex IHC Analysis

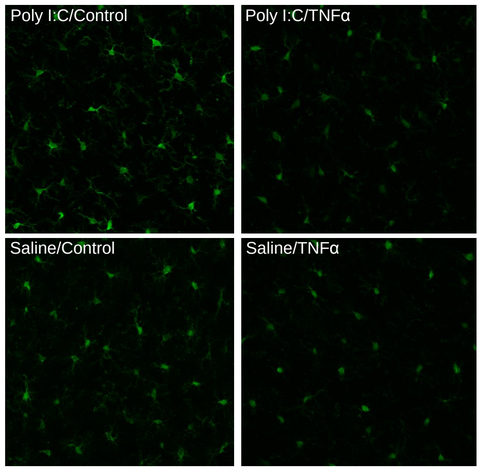


Figure 2. Hippocampus IHC Analysis

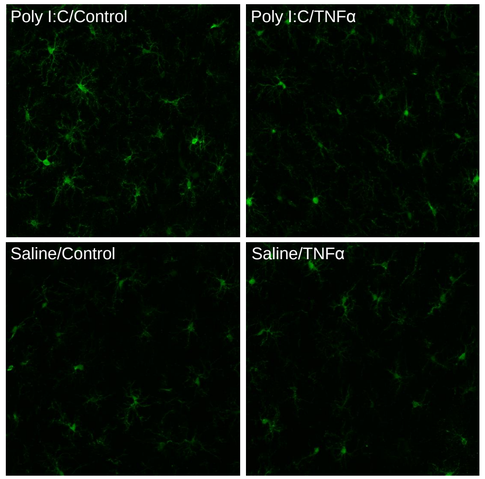
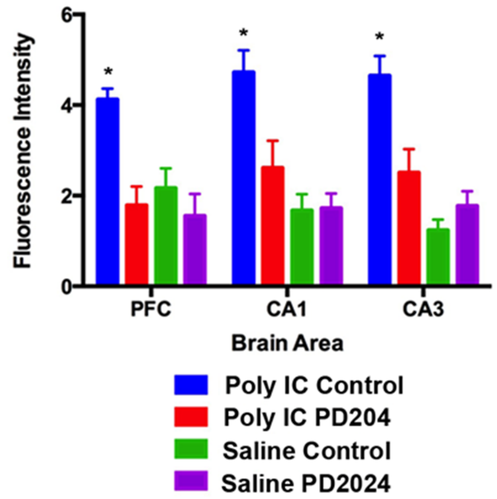


Figure 3. Sampled Cell Body Fluorescence



- Across all three brain areas, the Poly IC Control demonstrated increased microglia cell body fluorescence intensity (*p<.05).
- Regardless of the brain area, rats administered PD2024 through the diet had decreased microglia activation levels similar to the saline controls.

Conclusions

- Treatment with poly I:C activates the neuroinflammatory response, indicated by elevated microglia levels.
- PD2024 is effective in reducing microglia activation through the modulation of TNFα.
- PD2024 treatment was similar to the control group (Neonatal Saline/Control).
- TNFα modulation could reduce the dose-dependent side effects observed with current antipsychotic medication and modulate the neuroinflammation in those diagnosed with SCHZ.
- Future studies will examine additional TNFα modulators produced by collaborators at P2D Bioscience, Inc. (Cincinnati, OH) to determine efficacy across multiple compounds in the Poly I:C model of schizophrenia.

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