Apr 12th, 9:20 AM - 9:35 AM

The Effect of Two Novel Anti-Inflammatory Drugs on Sensorimotor Gating and Microglial Activation in the Poly I:C Rodent Model of Schizophrenia

Heath W. Shelton  
*East Tennessee State University*

W. Drew Gill  
*East Tennessee State University*

Prasad Gabbita  
*P2D Bioscience, Inc.*

Russell W. Brown  
*East Tennessee State University*

Follow this and additional works at: [https://dc.etsu.edu/asrf](https://dc.etsu.edu/asrf)
The Effect of Two Novel Anti-Inflammatory Drugs on Sensorimotor Gating and Microglial Activation in the Poly I:C Rodent Model of Schizophrenia

Heath W. Shelton¹, W. Drew Gill², S. Prasad Gabbita³, Russell W. Brown²

¹ Department of Biological Sciences, College of Arts & Sciences, East Tennessee State University, Johnson City, TN.
² Department of Biomedical Sciences, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN.
³ P2D Bioscience, Inc., Cincinnati, OH.
Introduction
What is Schizophrenia (SCZ)?

• Chronic & debilitating neurobehavioral disorder

• Affects estimated 21 million people worldwide (WHO Statistics Sheet, 2018)

• Age at onset observed in adolescence or early adulthood

• Diagnosis based on clinical observation or self-reporting (Gejman et al., 2010)

• Costs U.S. approx. $62 billion annually for medications & other therapeutic expenses
Current Treatment

• Antipsychotic Medications
  • Typical – dopamine D₂ antagonists (e.g. Haloperidol)
    • Attempts to treat associated positive symptoms
  • Atypical – dopamine D₂ antagonists; act on histamine, norepinephrine, & serotonin (e.g. Clozapine, Olanzapine, & Risperidone)
    • Attempts to treat associated positive & negative symptoms

• Psychosocial Interventions
  • Individual & family therapy, social skills training, and vocational rehabilitation
Problems with Current Treatment

• Typical antipsychotic drugs (FGA)
  • Not designed to treat (-) symptoms
  • Potent extrapyramidal motor side effects

• Atypical antipsychotic drugs (SGA)
  • Dose-dependent side effects (Solmi et al., 2017)
    Weight gain, insulin resistance/diabetes, cognitive impairment,
    agranulocytosis, seizures, pneumonia, myocarditis, &
    hypersalivation.
  • Short time to discontinuation
Neuroinflammatory Aspect

- SCZ patients shown to have **increased inflammation in CNS** (Howes & McCutcheon, 2017; Van Kesteren et al., 2017)
Tumor Necrosis Factor-alpha (TNFα)

• Pro-inflammatory cytokine

• Implicated in some autoimmune diseases (ex. RA)

• Influence state of CNS defense cells, called microglia
Microglial Cells

• Primary immune cells of the CNS & scan local environment for cellular stress (Nimmerjahn et al., 2005)

• Normally exist as anti-inflammatory, neuroprotective agents (M2 state)

• Upon activation by TNFα secretion, switch to M1 state, which is pro-inflammatory & neurotoxic

• Activated M1 microglia leads to overexpression of pro-inflammatory cytokines (ex. TNFα) & ROS, resulting in synaptic loss & neuronal death (Howes & McCutcheon, 2017)
P2D Bioscience, Inc.
TNFα Modulator Development

• Isoindoline-derived compounds
  • PD2024 & PD340

• Small, anti-TNF molecules
  • **Destabilizes TNFα mRNA**
    • Decreases TNFα protein release & secretion

• Centrally-acting, anti-neuroinflammatory properties
  • Safe & well tolerated in small (rats) and large (dogs) animals

PD2024
$M_w = 179.0 \text{ g/mol}$
TNFα IC$_{50} = 3 \mu\text{M}$
Polyinosinic:polycytidylic Acid (Poly I:C)

- Immunostimulant
- Interaction with TLR3
- Synthetic dsRNA (virus-like)
- Activates innate immune system
- Mimics neonatal infection in humans

Poly I:C Rodent Model of SCZ

- **Behavioral deficits/neuropathology consistent with SCZ**
  - Sensorimotor gating
  - Cognitive
  - Dopamine hyperfunction
  - Structural abnormalities (cortical volume reduction in PFC & HPC) (Meyer, 2014)

- Symptoms emerge in offspring, reflects delayed onset as seen in humans

- Clozapine & Risperidone alleviate deficits in neonatally treated poly I:C rats
Hypotheses

1. Treatment with Poly I:C will increase TNFα protein levels similar to the neuroinflammatory response for individuals diagnosed with schizophrenia

2. Novel TNFα modulators will alleviate sensorimotor gating deficits of the rodent Poly I:C model

3. Novel TNFα modulators will reduce associated neuroinflammation via a decrease in microglial cell activation levels in the HPC and PFC, two brain areas that mediate sensorimotor gating
Experimental Design
Study Design: Experiment 1 – TNFα Protein Levels

• A total of 17 male Sprague-Dawley pups IP injected with either Poly I:C (2 mg/kg) or saline (0.9% NaCl) from P5-7

• Sacrificed at P30 (in accordance with Exp. 2 & 3 dietary manipulation)

• PFC & HPC dissected away

• Tissue subjected to TNFα ELISA kit (Biomatik, Inc.; Wilmington, DE)
  • Protein detection via colorimetric detection (450 nm)
Study Design: Experiment 2 – PD2024

Grouping & Conditions

• SD pups divided equally into 4 groups

(Poly I:C/Control, Poly I:C/PD2024, Saline/Control, Saline/PD2024)

• Poly I:C groups IP injected with Poly I:C (2 mg/kg) from P5-7

• Saline groups IP injected with saline (0.9% NaCl) from P5-7

• All animals weaned at P21, dietary manipulation began at P30

• PD2024 groups received PD2024 until P67

• Control groups received a normal diet until P67
Study Design: Experiment 3 – PD340

Grouping & Conditions

• SD pups divided equally into 6 groups

(Poly I:C/Control, Poly I:C/PD340 – 10 mg/kg, Poly I:C/PD340 – 30 mg/kg, Saline/Control, Saline/PD340 – 10 mg/kg, Saline/PD340 – 30 mg/kg)

• Poly I:C groups IP injected with Poly I:C (2 mg/kg) from P5-7

• Saline groups IP injected with saline (0.9% NaCl) from P5-7

• All animals weaned at P21, dietary manipulation began at P30

• PD340 groups received PD340 (10 mg/kg or 30 mg/kg) until P67

• Control groups received a normal diet until P67
Study Design: Experiments 2 & 3

Prepulse Inhibition (PPI)

• Behaviorally tested on PPI
  • During adolescence (P44-46) and adulthood (P60-66)

Sacrifice & Immunohistochemistry (IHC)

• Sacrificed at P67, PFC & HPC dissected away, subjected to IHC

• IHC examined microglial cell activation using the Iba1-GFP conjugated antibody system
Prepulse Inhibition (PPI)

Used to measure auditory sensorimotor gating

Image from: https://en.wikipedia.org/wiki/Prepulse_inhibition#/media/File:Prepulse_Inhibition_schematically.png
Immunohistochemistry (IHC)

Detection technique, selectively identifies protein(s) in cells

Image from Exp. 3
Results
Figure 1. TNFα Protein Levels Following Saline or Poly I:C Administration Between P33-35.
Figure 2. Experiment 2: PPI Performance in Adolescents and Adults.
Figure 3. Experiment 2: Microglial Cell Activation in the PFC and HPC.
Figure 4. Experiment 2: Representative Images of Iba1-GFP Labeled Microglia Cells in the PFC.
Figure 5. Experiment 3: PPI Performance for Adolescents and Adults.
Figure 6. Experiment 3: Microglial Cell Activation in the PFC and HPC.
Conclusions & Future Directions
Conclusions

• Neonatal Poly I:C resulted in behavioral deficits in adolescence & adulthood, consistent with clinical observation & diagnosis of SCZ.

• Two novel TNF-alpha modulators (PD2024 & PD340) alleviated sensorimotor gating deficits in adolescence and adulthood.
  • Decreased microglial cell activation known to mediate sensorimotor gating
• PD2024 and PD340 adjunctively used with antipsychotic drugs in the Poly I:C and other rodent models of SCZ.
Acknowledgements

Thesis Committee
Dr. Russell Brown (Chair)
Dr. Gregory Ordway
Dr. Donald Hoover

P2D Bioscience, Inc.
Dr. Prasad Gabbita

GPSA
David Moore (GPSA Advisor)
GPSA membership

School of Graduate Studies
Dr. Sharon McGee
Dr. Karin Bartoszuk
Emily Redd

Graduate Students
Drew Gill
Rudy Chapman
Kyle Travis
Amanda Smith
Trevor Chapman

Brown Lab

SCHOOL of GRADUATE STUDIES

Behavioral Neuroscience Laboratory
East Tennessee State University

GPSA
GRADUATE & PROFESSIONAL STUDENT ASSOCIATION
Questions?