Efficacy of Fluoxetine and Resveratrol for Easing Memory and Mood Dysfunction in an Animal Model of Gulf War Illness

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Gulf War Illness (GWI)

Affected Population

~33% of 700,000 Veterans who served in the 1991 Persian Gulf War-I (PGW-I)

Symptoms

Chronic multi-symptom health problem with multiple CNS impairments:

- Learning and memory impairments
- Depression and anxiety
- Sleep problems
- Pain etc.

Potential Causes

(1) Intake of pyridostigmine bromide (PB)
(2) Exposure to Pesticides such as DEET or permethrin (PM)
(3) Low-level exposure to nerve gas agents, exposure to oil well fire smoke etc.

Multiple chemical exposure hypothesis

Emerged as one of the most likely causes of GWI

Concurrent exposure to chemicals PB, DEET and PM (with or without stress)
Rat Model of Gulf War Illness (GWI)

Generated through exposing rats to low doses of PB (1.3 mg/Kg), DEET (40mg/Kg), permethrin (0.13 mg/Kg) with or without 5 minutes of restraint stress for four weeks

Pathophysiology

*Cognitive and Mood Dysfunction associated with Hippocampus pathology*

- Decreased levels of hippocampus neurogenesis – one of the substrates important for cognitive and mood function
- Partial loss of hippocampal principal neurons
- Mild inflammation (reactive astrocytes and activated microglia)

Oxidative Stress

Therapeutic Strategies Examined

*Administration of antidepressant Fluoxetine or an anti-inflammatory and antioxidant compound Resveratrol*

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Cognitive Impairment in a Rat Model of GWI (Water Maze Test)

*Parihar, Hattiangady, Shuai and Shetty, Neuropsychopharmacology, 38:2348-2362, 2013*
Cognitive Impairment in a Rat Model of GWI
(Object Location and Recognition Tests)

Object Location Test

Novel Object Recognition Test

Mood Impairments in a Rat Model of GWI

Novelty Suppressed Feeding Test

Forced Swim Test

Voluntary Exercise Test
(In Cages Fitted with Running wheels)

Hattiangady, Mishra, Kodali, Shuai, Rao and Shetty, Frontiers in Behav Neurosci, 8:78, 2014

Parihar, Hattiangady, Shuai and Shetty, Neuropsychopharmacology, 38:2548-2562, 2013

Hattiangady, Mishra, Kodali, Shuai, Rao and Shetty, Frontiers in Behav Neurosci, 8:78, 2014
Hippocampus Neurogenesis Impairments in a Rat Model of GWI

Parihar, Hattiangady, Shuai and Shetty, Neuropsychopharmacology, 38:2348-2362, 2013

Stem Cell Proliferation Impairments in a Rat Model of GWI

Parihar, Hattiangady, Shuai and Shetty, Neuropsychopharmacology, 38:2348-2362, 2013
Hippocampal Neuron Loss in a Rat Model of GWI

Reactive Glial Cells in a Rat Model of GWI

Activated Microglia (ED-1+)

Reactive Astrocytes

Oxidative Stress in a Rat Model of GWI

Expression of oxidative stress related genes in the hippocampus (measured via qRT-PCR) ~6 months after exposure to GWI-related chemicals and stress

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Efficacy of Fluoxetine Treatment in a Rat Model of GWI

Rational for choosing fluoxetine (Prozac):
- Most commonly used antidepressant
- Mood impairment is one of the conspicuous symptoms in GWI and in animal models of GWI
- Fluoxetine is known to improve hippocampus neurogenesis in a variety of animal models
- FLU is a SSRI, and hence can increase serotonin levels
- Increased serotonin levels in the hippocampus are associated with increased levels of BDNF and enhanced hippocampus neurogenesis

Both increased neurogenesis and BDNF levels in the hippocampus may improve cognitive and mood function

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Research Design

- Adult SD Rats (~4 months old)
- Exposure to GWIR-Chemicals and 5 min Restraint Stress
  - PB, 1.3 mg/Kg, DEET, 40 mg/Kg, PM, 0.13 mg/Kg; Daily for 4 weeks
- Vehicle (SQ, Daily for 4 weeks) [BrdU, last 12 days]
- Fluoxetine (10 mg/Kg, SQ, daily for 4 weeks) [BrdU, last 12 days]
- A month of Waiting Period
- Behavioral Tests (WMT, OLT, NORT, FST)
- Euthanasia and Histological Analyses
Fluoxetine Improves Spatial Learning and Memory Function in a Rat Model of GWI (evidenced through WMT)

Two-way RM-ANOVA: Naive versus GWI-VEH, p<0.05
GWI-VEH versus GWI-FLU, p<0.05; Naive versus GWI-FLU, p>0.05.

Fluoxetine Normalizes Object Location Memory Function in a Rat Model of GWI (evidenced through OLT)

a) Habituation phase
b) Sample phase
1 hour
Object 2
Object 1
c) Testing phase
1 hour
Object 2
Object 1
Naive
GWI-VEH
GWI-FLU

Object Location Discrimination Index
Fluoxetine Normalizes Novel Object Recognition Memory Function in a Rat Model of GWI (evidenced through NORT)

Fluoxetine Reduces Depressive-like Behavior in a Rat Model of GWI (evidenced through FST)
**Fluoxetine Improves Net Hippocampus neurogenesis in a Rat Model of GWI**

Fluoxetine Enhances the Generation of New Dentate Granule Cells (Doublecortin+ Cells in the Hippocampus) in a rat Model of GWI

(Even after the termination of treatment)
CONCLUSIONS

Efficacy of Fluoxetine for Easing Cognitive and Mood Dysfunction in GWI

Fluoxetine treatment to rats exposed to Gulf War Illness-related chemicals and stress:
- Normalizes hippocampus-dependent spatial learning and memory function
- Alleviates hippocampus-dependent object location memory dysfunction
- Normalizes novel object recognition memory function
- Eases mood dysfunction
- Enhances neurogenesis in the hippocampus

Considering the purported functions of hippocampal neurogenesis, increased neurogenesis at least partially underlies the beneficial effects mediated by fluoxetine. However, other mechanisms may be involved – need further studies!

Efficacy of Resveratrol Treatment in a Rat Model of GWI

Resveratrol (RESV), a naturally occurring polyphenol found in skin of red grapes, red wine and some nuts

Rational for choosing Resveratrol:
RESV has the ability for:
- Up-regulating SIRT1 (a longevity gene vital for maintenance of normal cognitive function and synaptic plasticity)
- Modulating inflammation
- Reducing Oxidative Stress

As our animal model of GWI exhibits inflammation as well as oxidative stress in the hippocampus, RESV appeared appropriate for easing symptoms of GWI.
RESVERATROL Improves Spatial Learning and Memory Function in a Rat Model of GWI (evidenced through WMT)

Two-way RM-ANOVA: Naïve versus GWI-VEH, p<0.05; GWI-VEH versus GWI-RESV, p<0.05; Naïve versus GWI-RESV, p>0.05.

Two-way RM-ANOVA: Naïve versus GWI-VEH, p<0.05; GWI-VEH versus GWI-RESV, p<0.05; Naïve versus GWI-RESV, p>0.05.

Memory Retrieval (Probe) Test

RESVERATROL Reverses Object Location Memory Dysfunction in a Rat Model of GWI (evidenced through OLT)

a) Habituation phase
b) Sample phase
1 hour

Object 2
Object 1

Object 2
Object 1

c) Testing phase

Naive
GWI-VEH
GWI-RESV

Object Location Discrimination
0 40 80

Object Location Discrimination
0 40 80

Object Location Discrimination
0 40 80

Naive
GWI-VEH
GWI-RESV
RESVERATROL Normalizes Novel Object Recognition Memory Function in a Rat Model of GWI (evidenced through NORT)

RESVERATROL Ease Depression in a Rat Model of GWI (evidenced through FST)
RESVERATROL Improves Net Hippocampus Neurogenesis in a Rat Model of GWI

Newly Born Cells (BrdU+) in the DG

Newly Born neurons that mature into NeuN+ dentate granule cells

Net Hippocampus Neurogenesis

RESVERATROL Enhances the Generation of New Dentate Granule Cells in a rat Model of GWI (even after the termination of treatment)
RESVERATROL Suppresses Hippocampus Inflammation in a Rat Model of GWI

Dentate Gyrus

CA3 Region

Fimbria

RESVERATROL Modulates Oxidative Stress in a Rat Model of GWI

Expression of oxidative stress related genes in the hippocampus (measured via qRT-PCR)

GWI+RESV versus GWI, p<0.05-0.0001 (Psmb5, Mpo, Ercc2, Gpx2, Tpo, Gpx2, Gpx4, Prdx6, Gsr, Gpx1, Srxn1, Gpx7, Sod3)

GWI+RESV versus VEH Control, p<0.01-0.00001 (Psmb5, Mpo, Ercc2, Gpx2, Tpo, Gpx2, Gpx4, Prdx6, Gsr, Gpx1, Srxn1, Gpx7, Sod3)
CONCLUSIONS

Efficacy of RESVERATROL for Improving Cognitive and Mood Function in GWI

Resveratrol treatment to rats exposed to Gulf War Illness-related chemicals and stress:

- Improves hippocampus-dependent spatial learning and memory function
- Relieves hippocampus-dependent object location memory dysfunction
- Normalizes novel object recognition memory function
- Reverses mood dysfunction
- Increases neurogenesis in the hippocampus
- Suppresses inflammation in the hippocampus
- Modulates oxidative stress in the hippocampus

It is likely that improved neurogenesis, decreased levels of inflammation and oxidative stress mediated by Resveratrol contributed to the beneficial effects observed in this study.

However, other mechanisms (e.g., activation of SIRT1) may also be involved – Need further studies!

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