SIRT1 Activator Treatment of Autoimmune and Viral-Induced Optic Neuritis

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Financial Disclosure

- Funding for the studies presented were provided in part by NIH/NEI, National Multiple Sclerosis Society, Research to Prevent Blindness, F. M. Kirby Foundation, and Sirtris Pharmaceuticals, a GSK company.

- SIRT1 activating compounds were provided by Sirtris.

- I have received honoraria for talks presented at Sirtris.
Overview

- Review of optic neuritis
- EAE model of MS/optic neuritis
- Neuroprotection by SIRT1 activators in EAE
- Viral Induced optic neuritis
Optic Neuritis

- Inflammatory demyelinating disease of the optic nerve
- Either idiopathic or associated with multiple sclerosis (MS)

Presentation: Acute unilateral vision loss
- Progresses 1-2 weeks
- Pain on eye movement
Optic Neuritis: Outcomes

- Vision recovers (95% ≥ 20/40) over several wks with or without treatment
- Vision recovers faster with steroids
- Steroids do NOT change final visual acuity
- Approx. 40-60% have some permanent visual loss:
  - 40% decreased acuity
  - 38% contrast sensitivity
  - 20% visual field defects
  - 35% color vision

Optic Neuritis: Neuronal Loss

- Significant retinal ganglion cell (RGC) axonal loss occurs following optic neuritis

- Retinal nerve fiber layer (NFL) thinning on OCT or scanning laser polarimetry


- NFL thinning correlates with decreased vision
Optic Neuritis: Vision Loss

- Acute vision loss from inflammation and demyelination
  - Improves when inflammation resolves

- Permanent vision loss from death of RGCs
  - No improvement after inflammation
Studying Neuroprotection in Optic Neuritis: Why? How?

- **Goal:** Prevent permanent vision loss
- **Means:** Prevent RGC death

- **Other important benefits:**
  - Potential neuroprotection for other MS lesions
  - Identify candidate neuroprotective therapies for other RGC diseases
    - Glaucoma is slowly progressive, difficult to evaluate outcomes
    - Short time frame of optic neuritis will allow shorter clinical trials with measurable vision and imaging parameters

- **Methods:** Use animal models of optic neuritis to identify drugs that prevent RGC loss
EAE: Animal model of MS

- Experimental autoimmune encephalomyelitis (EAE)
- Most widely used animal model of MS
- Animals injected with myelin proteins
- Develop inflammation in brain, spine, and optic nerve
- Histopathology similar to MS
- Different strains and antigens can be used to produce different clinical courses
Optic Neuritis in Relapsing/Remitting EAE

- SJL/J mice immunized with proteolipid protein

- Optic neuritis induced in 60-70% of eyes

RGC loss in EAE optic neuritis

- RGCs labelled by fluorogold injection into superior colliculi
- RGC loss detected by day 14 after EAE induction

![FG labeled RGCs](image)

*Multiple Sclerosis*
12:526-32
Axonal loss in EAE optic neuritis

Exp Eye Res 2008;87:208-13
Axonal and neuronal loss occur by d14 post-immunization in R/R EAE

Can we use this model to identify neuroprotective therapies?
Potential Neuroprotective Agents: Activators of SIRT1 Gene Family

- NAD-dependent histone deacetylases
- Regulate gene expression and promote increased lifespan
- Deacetylation of transcription factors, apoptosis proteins and structural proteins

From Porcu and Chiarugi (2005)
TRENDS in Pharmacol. Sci. 26:94-103
**SIRT1 Activators**

- Increase SIRT1 affinity for protein targets
- At least 18 identified
- Resveratrol, a sirtuin activator found in red wine, prevents degradation of axotomized DRG neurons

Hypothesis: SIRT1 activation is neuroprotective for RGCs during acute optic neuritis

Approach: Examine effects of SIRT1 activators on RGC survival in R/R EAE
Intravitreal SRT501 attenuates RGC loss in EAE optic neuritis

- SRT501 is a pharmaceutical grade formulation of resveratrol
- Intravitreal SRT501 prevents RGC loss during optic neuritis
- SRT501 given intravitreally on d0, 3, 7, 11; RGCs counted d14
SRT501 does not prevent EAE or optic nerve inflammation

![Graph showing clinical EAE score versus days post-immunization for Placebo and SRT501 at concentrations of 6 μM, 13 μM, and 100 μM. The graph demonstrates an increase in clinical EAE score over time for all treatments compared to Placebo.]

![Bar chart showing incidence of optic neuritis (%) for Placebo and SRT501 at concentrations of 6 μM, 13 μM, and 100 μM. The chart indicates a higher incidence of optic neuritis for SRT501 compared to Placebo.]

**Clinical EAE Score**
- Placebo
- 6 μM SRT501
- 13 μM SRT501
- 100 μM SRT501

**Days Post-Immunization**
0 2 4 6 8 10 12 14

**Incidence of Optic Neuritis (%)**
0 25 50 75 100
Sirtinol, a SIRT1 inhibitor, blocks the neuroprotective effect of SRT501

Question:
Does mechanism of SRT501 neuroprotection work through SIRT activation?
Single dose of SRT501 is neuroprotective at d14 and d30

Questions: What timing of SRT501 treatment is sufficient for neuroprotection?
Is RGC loss merely delayed, or is there longer-term neuroprotection?
Intravitreal SRT501 Results

- SRT501 prevents RGC loss during acute optic neuritis in a dose-dependent manner.
- SRT501 effects are mediated by SIRT1 activation.
- Intravitreal SRT501 is not toxic to RGCs.
- Intravitreal SRT501 does not prevent optic nerve inflammation or EAE.

Question: Can oral therapy prevent RGC loss and other neurodegeneration in EAE?
Oral SRT501 Treatment Beginning Prior to Optic Neuritis Attenuates Acute RGC Loss at Day 14

Mice treated with SRT501 daily from d8-14 by oral gavage.

Sacrificed d14.

SRT501 prevented RGC loss in dose-dependent manner.
Oral SRT501 Treatment Beginning After Optic Neuritis Attenuates Acute RGC Loss at Day 14

Mice Sacrificed day 14:

1000 mg/kg SRT501 daily from d10-14 by oral gavage prevented RGC loss
Oral SRT501 Attenuates Longer-Term RGC Loss at Day 30

Mice Sacrificed day 30:

1000 mg/kg SRT501 daily from d10-14 by oral gavage prevented RGC loss

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>RGC Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls - vehicle</td>
<td>N = 10</td>
<td></td>
</tr>
<tr>
<td>EAE - vehicle</td>
<td>N = 22</td>
<td></td>
</tr>
<tr>
<td>EAE - 1000 mg/kg SRT501</td>
<td>N = 26</td>
<td></td>
</tr>
</tbody>
</table>

EAE - 1000 mg/kg SRT501 daily from d10-14 by oral gavage prevented RGC loss.
SRT501 preserves axons as well as RGC bodies

SRT501 does not suppress optic nerve inflammation
Sirtinol blocks the neuroprotective effect of oral SRT501 on RGCs
Oral SRT501 Suppresses Residual EAE Symptoms During Remission

SRT501 treated mice had better recovery from first clinical episode of EAE than vehicle treated mice.

![Graph showing EAE Scores with treatment effects](image-url)
SRT501 preserves spinal cord axons without suppressing inflammation
Examination of Spinal Cord and Optic Nerve Inflammatory Cells Following SRT501 Treatment

Mononuclear cells isolated from spinal cords and optic nerves of EAE mice

No significant difference in number of mononuclear cells isolated from SRT501- vs vehicle-treated EAE spinal cords
Spinal Cord Inflammatory Cells Following SRT501 Treatment

No difference in type of mononuclear cells isolated from SRT501- vs vehicle-treated EAE spinal cords analyzed by flow cytometry
SRT1720 is also neuroprotective for RGCs and spinal cord axons.
Oral SIRT1 Activator Summary

- SIRT1 activators prevent RGC loss during acute optic neuritis in a dose-dependent manner
- SIRT1 activators reduce residual neurological dysfunction during remission in R/R EAE
- Mechanism does not involve suppression of inflammation
- Therefore, treatment of MS with SIRT1 activators may provide added benefits to current immunomodulatory therapies
- Experimental optic neuritis in EAE is a useful model for examining neuroprotective therapies
Oral SIRT1 Activators in R/R EAE - Limitations

- SJL mice have inherent retinal photoreceptor degeneration
  - Cannot test visual function

- Optic neuritis detected by histology
  - Can only be identified after sacrifice
  - Inflammation varies, can be missed if focal or if resolved
  - Need in vivo methods to identify eyes with optic neuritis

- Etiology of optic neuritis/MS not fully understood
  - Autoimmune
  - ? Genetic component
  - ? Viral trigger

- Need to use additional optic neuritis models
Viral-Induced Model of MS

Neurovirulent strains of mouse hepatitis virus (MHV) induce encephalitis and demyelination in C57/Bl6 mice following intracranial inoculation.

MHV-A59 induces demyelination

MHV2 induces meningitis, without encephalitis or demyelination

J Virol 2008;82:8882-6
Viral-Induced Model of MS

Similar effects are seen in spinal cord

MHV-A59 induces meningitis, myelitis and demyelination

MHV2 induces meningitis, without myelitis or demyelination

J Virol 2008;82:8882-6
Viral-Induced Experimental Optic Neuritis

MHV-A59 induces optic neuritis, whereas MHV2 does not

J Virol 2008;82:8882-6
Viral-Induced Experimental Optic Neuritis

MHV-induced optic nerve inflammation consists predominantly of macrophages/microglia
Viral-Induced Experimental Optic Neuritis

- Optic neuritis occurs in this viral-induced MS model
- Demyelinating strains (MHV-A59), but not non-demyelinating strains (MHV2) induce optic neuritis
- Inflammation differs from EAE
  - EAE: T cell mediated
  - MHV: Macrophage mediated
- SIRT1 activator mediated neuroprotection can evaluated in distinct models of optic neuritis
Conclusions

- SIRT1 activators prevent axonal damage and neuronal loss in EAE optic neuritis
- SIRT1 activators do not reduce inflammation, and may act downstream of inflammation-induced axonal injury
- SIRT1 activators represent a potential new treatment for MS/optic neuritis that may provide added benefits to current immunomodulatory therapies
Acknowledgments

Collaborators:  

**TJU**  
A. M. Rostami  
Sirtris  
Elvira Ventura  
Peter Elliott  
Yangtai Guan  
Jim Ellis  
Philomela Tabuena  

**U Penn**  
Zoe Kelly  
Reas Sulaimankutty  
Mayssa Nasrallah  
Mahasweta Dutt  
Mira Sachdeva  
Jean Bennett  
Daniel Chung  
Karen Revere  
Tonia Rex  
Mark Consugar  
Gui-Shuang Ying  
Vivian Lee  
Kimberly Dine

Queens-Belfast  
Denise Fitzgerald

IISER-Kolkata  
Jayasri Das Sarma

This work was supported by the NIH/NEI; RPB; the NMSS; Sirtris, a GSK Company; and the F. M. Kirby Foundation.
Thank you!