3H-1, 2-Dithiole-3-Thione (D3T) as a Novel Therapeutic Agent for the Treatment of Experimental Autoimmune Encephalomyelitis

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Disclosure Information

• All authors declare that there are no conflicts of interest.

Learning Objectives

• Examine the potential beneficial effect of D3T in the treatment of EAE, an animal model of MS.

• Investigate the molecular mechanisms underlying the protective effect of D3T in EAE.

Multiple Sclerosis (MS)

• A chronic autoimmune, inflammatory neurological disease of central nervous system (CNS).
  * More then 2.3 million people affected by MS worldwide.
    - National Multiple Sclerosis Society website.

• Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model for MS.
**3H-1,2-Dithiole-3-Thione (D3T)**

- D3T, a natural compound, can be found in cruciferous vegetables.
- D3T is the parent compound of dithiolethiones, which is a well-known class of cancer chemopreventive agents.
  - Mol Cancer Ther (2008)

- D3T provides antioxidant activity through Nrf2 pathway.

- Nrf2 pathway has been reported to provide anti-inflammatory effects.
  (Nrf2 / HO-1 / NF-κB pathway)

**Hypothesis**

The effect of D3T in the autoimmune inflammatory disease MS/EAE is unknown

D3T → Nrf2 → anti-inflammation

To investigate whether D3T would provide a beneficial effect in EAE through its anti-inflammatory property

**D3T Reduces EAE Severity**

<table>
<thead>
<tr>
<th>Clinical scores</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No obvious changes.</td>
</tr>
<tr>
<td>1</td>
<td>Limp tail.</td>
</tr>
<tr>
<td>2</td>
<td>Limp tail and weakness of hind legs.</td>
</tr>
<tr>
<td>3</td>
<td>Limp tail and partial paralysis of hind legs.</td>
</tr>
<tr>
<td>4</td>
<td>Limp tail, complete hind leg and partial front leg paralysis.</td>
</tr>
<tr>
<td>5</td>
<td>Mouse is found dead due to paralysis.</td>
</tr>
</tbody>
</table>

C57BL/6 female
s.c. MOG immunization
i.p. D3T 10mg/kg
Every day starting from day 1
Stop Treatment
Clinical scores

Days post immunization

**Clinical scores**

- Vehicle
- D3T

*p<0.05; **p<0.01; ***p<0.001
**D3T Inhibits EAE Progression**

- EAE mice
  - Disease score 1.5-2
- i.p. D3T 30mg/kg
  - Every other day
- Clinical scores

**Pathogenesis of MS/EAE**

- Periphery
- BBB
- CNS
- Neuron

**D3T Inhibits DC Activation**

- BM-DCs / FACS
  - CD80, CD86
  - IL-23p19, IL-12p35, IL-12p40
- BM-DCs / mRNA
  - IL-23, IL-12
- BM-DCs / Protein
  - IL-12
- BM-DCs / WB
  - Nrf2
- BM-DCs / ELISA
  - IL-23, IL-12

**Nrf2 pathway → anti-inflammation**
**Pathogenesis of MS/EAE**

**Periphery**

- D3T
- T cells
- DCs
- Cytokines

**BBB**

- Chemokines
- IFN-γ
- IL-17

**CNS**

- Neuron
- Myelin sheath
- Cytokines

**Neuron**

- Peripheral lymphoid organ
- D3T
- Th1
- Th17
- MGl

**MG**

- Microglia
- Blood-brain barrier

**DCs**

- Dendritic cells
- Peripheral lymphoid organ

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**D3T Inhibits Th1 and Th17 Differentiation**

- CD4+ T cells
- CD4+ IFN-γ
- CD4+ IL-17

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**D3T Reduces Peripheral Th1/Th17 Differentiation in EAE**

- Vehicle or D3T-treated C57Bl/6 EAE mice
- Th1, Th17
- CD4+ IFN-γ
- CD4+ IL-17

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**D3T Decreases CNS Th1/Th17 Infiltration in EAE**

- Vehicle or D3T-treated C57Bl/6 EAE mice
- Th1, Th17
- CD4+ IFN-γ
- CD4+ IL-17
Pathogenesis of MS/EAE

**Periphery**
- DCs: dendritic cells
- MG: microglia
- BBB: blood-brain barrier

**CNS**
- Neuron
- Chemokines
- Cytokines
- Myelin sheath

**Neuron**
- Cytokines
- Chemokines

D3T Inhibits MG Activation

- pMG: Primary microglia generated from neonatal mouse brain
- Ramified form (resting)
- Amoeboid form (activated)

**D3T Inhibits MG Activation in the CNS of EAE Mice**

- Mononuclear cells / FACS
- CD80^+ CD45^- CD11b^+
- CD86^+ CD45^- CD11b^+
- CD80^+ MG
- CD86^+ MG

**D3T Suppresses MG Activation in the CNS of EAE Mice**

- Mononuclear cells / FACS
- CD80^+ CD45^- CD11b^+
- CD86^+ CD45^- CD11b^+
- CD80^+ MG
- CD86^+ MG

**D3T Inhibits MG Activation**

- IL-23p19
- IL-12p35
- IL-12p40
- iNOS
- Gm-csf
- Il-1β
- Il-6

**D3T Suppresses MG Activation**

- **p<0.05, ***p<0.001**

**Inhibits MG Activation**

- D3T suppresses microglia activation in the CNS of EAE mice.
D3T Reduces Inflammatory Cytokine Expression in the CNS of EAE

**Conclusion**

**Acknowledgments**

**The study is supported by Anna Yoder MS Fund and AHA**
ALT: alanine transaminase

Dimethyl fumarate (DMF)

C<sub>6</sub>H<sub>8</sub>O<sub>4</sub>
MW : 144.127 g/mol

3H-1, 2-dithiole-3-thione (D3T)

C<sub>3</sub>H<sub>2</sub>S<sub>3</sub>
MW : 134.243 g/mol

IL-23p19 / β-actin
LPS 3hMED
D3T
Vehicle
DMF

IL-12p35 / β-actin
LPS 3hMED
D3T
Vehicle
DMF

IL-12p40 / β-actin
LPS 3hMED
D3T
Vehicle
DMF

Nrf2+/−, Nrf2−/− female

Lp. D3T 30mg/kg Every other day starting from day 3

Clinical scores

Days post immunization

<table>
<thead>
<tr>
<th>Vehicle-Nrf2+/−</th>
<th>Vehicle-Nrf2−/−</th>
<th>D3T-Nrf2+/−</th>
<th>D3T-Nrf2−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>14/14</td>
<td>0/9</td>
<td>10/10</td>
</tr>
<tr>
<td>Mortality</td>
<td>1/14</td>
<td>0/9</td>
<td>0/10</td>
</tr>
<tr>
<td>Maximum score</td>
<td>3.9±0.2</td>
<td>1.5±0.3</td>
<td>3.7±0.4</td>
</tr>
<tr>
<td>Cumulative score</td>
<td>50.1±2.3</td>
<td>9.9±2.3</td>
<td>55.6±5.5</td>
</tr>
</tbody>
</table>

* P<0.05
D3T Inhibits DC Activation

Nrf2 pathway → anti-inflammation

CD4+ T cells / FACS

Nrf2+/- & Nrf2-/− BM-DCs / FACS

BM-DCs WB
Nrf2 Pathway

- Nuclear factor E2-related factor 2 (Nrf2) is a basic leucine zipper transcription factor.

Keap1: Kelch-like ECH-associated protein 1
ARE: Antioxidant Response Element
HO-1: Heme oxygenase 1
GCLC: Glutamate-cysteine ligase, catalytic subunit
NQO1: NAD(P)H dehydrogenase, quinone 1

Keap1: Kelch-like ECH-associated protein 1
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Vehicle- or D3T-treated C57BL/6 EAE mice

Splenocytes / FACS

Tregs

CD4+ Foxp3+

Vehicle (n=7) D3T (n=7)

Splenocytes / FACS

Vehicle

D3T

Vehicle

D3T

Splenocytes

BM-DCs / Q-PCR

IL-1β

GM-CSF

IL-6

MED LPS 2h LPS 6h MED LPS 2h LPS 6h MED LPS 2h LPS 6h

D3T Vehicle

D3T Vehicle

*p<0.05, ***p<0.001

***p<0.001

IL-27 → IL-10+ Tr1

TGF-β → Foxp3+ Tregs

***p<0.001
**BM-DCs / FACS**

**D3T Promotes Phase II Anti-oxidant Enzyme Induction in MG**

**pMG / Q-PCR**

**BV2 Cells**

*P<0.05, **P<0.01, ***P<0.001
D3T Promotes Phase II Anti-oxidant Enzyme Induction in MG

NQO1

GCLC

HO-1

*p<0.05; **p<0.01; ***p<0.001