Modulation of TRP channels by resolvins in mouse and human

Ru-Rong Ji, PhD

Pain Research Division
Department of Anesthesiology
Department of Neurobiology
Duke University Medical Center
Omega-3 polyunsaturated fatty acids

Dietary essentially fat

Omega-3 polyunsaturated fatty acids

Docosahexaenoic acid (DHA)

Eicosapentaenoic acid (EPA)

Powerful metabolic products (Resolvins)

Ji et al., *Trends Neurosci*, 2011
Chronic pain is a major health problem

- Over **100 million** Americans have chronic pain
- The total financial cost of is near **600 billion** a year in the US.
- Annual US expenditure on pain (medical costs and lost wages) is higher than that for **cancer**, **heart disease**, and **diabetes** combined.
- **Co-morbidity**: Chronic pain is associated with depression, anxiety, insomnia, etc.
- **Prevention** and treatment of this **epidemic** are equally important.

*Pizzo & Clark, N Engl J Med., 2012*  
American Pain Society
Peripheral mechanisms of pain

Peripheral nerve terminal of nociceptor neuron

Ji et al., Nat Rev Drug Disco., 2014
Central mechanisms of pain

Tissue injury & Nerve injury

Microglia Activation
- p38-P
- ERK-P

Injury
- TNF-α
- IL-1β
- BDNF

Astroglia activation
- JNK-P

Glial mediators

Primary afferents
- AMPA
- NMDA
- TrkB

Spinal cord dorsal horn neurons
- BDNF
- Glu
- CCL2
- IL-1β

Persistent pain

Excitation

Inhibition

Primary afferents

Spinal cord dorsal horn neurons

JNK-P

GABA-A-R

Gly-R

TNF-α

IL-1β
Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators

Charles N. Serhan*, Nan Chiang* and Thomas E. Van Dyke†

Serhan et al., 2008; Nature Reviews Immunology

Resolvin E1 (RvE1)

Resolvin D1 (RvD1)
Biosynthesis of resolvins from PUFA Precursors

**EPA**
- Aspirin:COX-2
- P450
- microbial

**DHA**
- Aspirin:COX-2
- LOX

**17R-Resolvin D Series**
- Acetylated COX-2

**17S-Resolvin D Series**
- Lipoxygenase mechanism

**RvD1**

**RvD2**

**RvE1**
### Resolvin are potent inhibitors of inflammation in various animal models

Table 1. Anti-inflammatory actions of resolvins in animal models of inflammation

<table>
<thead>
<tr>
<th>Resolvin</th>
<th>Animal</th>
<th>Inflammatory condition</th>
<th>Anti-inflammatory effects</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>RvE1</td>
<td>Mouse</td>
<td>Dorsal air pouch</td>
<td>Stops neutrophil recruitment</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peritonitis</td>
<td>Reduces neutrophil recruitment</td>
<td>[49,59]</td>
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<td>Regulates cytokine and chemokine expression</td>
<td>[49,59]</td>
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<td></td>
<td>Retinopathy</td>
<td>Alleviates neovascularization and reduces TNF-α expression</td>
<td>[61]</td>
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<td></td>
<td></td>
<td>Colitis</td>
<td>Decreases neutrophil recruitment and improves survival</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allergy</td>
<td>Resolves allergic airway inflammation</td>
<td>[63,64]</td>
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<td></td>
<td></td>
<td></td>
<td>Regulates NK cell migration</td>
<td>[63,64]</td>
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<tr>
<td></td>
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<td>Ocular lesion</td>
<td>Controls HSV-induced ocular inflammatory lesions</td>
<td>[65]</td>
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<td></td>
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<td>Increases IL-10 and decreases IL-6 expression</td>
<td>[65]</td>
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<td></td>
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<td>Lung injury</td>
<td>Protects against bacterial pneumonia and acute lung injury</td>
<td>[66]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Enhances clearance of bacteria</td>
<td>[66]</td>
</tr>
<tr>
<td>RvD1</td>
<td>Mouse</td>
<td>Kidney ischemia</td>
<td>Protects from ischemia–reperfusion-induced kidney damage</td>
<td>[67]</td>
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<tr>
<td></td>
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<td></td>
<td>Regulates macrophage function</td>
<td>[67]</td>
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<tr>
<td></td>
<td></td>
<td>Peritonitis</td>
<td>Stops neutrophil recruitment and modulates miRNAs</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dorsal skin air pouch</td>
<td>Stops neutrophil recruitment</td>
<td>[46]</td>
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<tr>
<td></td>
<td></td>
<td>Oxidative stress</td>
<td>Controls inflammation during oxidative stress</td>
<td>[69]</td>
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<tr>
<td>AT-RvD1</td>
<td>Mouse</td>
<td>TMJ inflammation</td>
<td>Protects inflammation in the TMJ</td>
<td>[70]</td>
</tr>
</tbody>
</table>

*Ji et al., Trends Neurosci, 2011*
Resolvins prevent CRG-induced heat hyperalgesia

- Vehicle
- RvD1, 20 ng (60 pmol)
- RvE1, 20 ng (60 pmol)

![Graph showing PWL (seconds) over time after CRG injection for different treatments.](image)

Xu et al., Nat Med, 2010
RvE1 inhibits CRG-induced inflammation

Paw edema
Neutrophil infiltration

Paw volume increase (% of BL)
MPO activity (U mg⁻¹ protein)

CRG  CRG+
(RvE1, 20ng)  

Naive  CRG  CRG+
(RvE1, 20ng)

*  

RvE1 inhibits CRG- induced inflammation
Neutrophil infiltration

Neutrophil infiltration
RvE1 inhibits cytokine/chemokine expression after inflammation

Cytokine array
Formalin-induced spontaneous pain

- Vehicle
- RvE1, 1 ng
- Morphine, 100 ng
- NS-398, 10 μg

Duration of licking/flinching (s)

Time (min) after formalin injection

Duration of licking/flinching (s)

Phase-1 (0-10 min)
Phase-2 (10-45 min)
Formalin-induced 2nd-phase pain

![Graph showing inhibition of phase-2 pain behavior (%) vs. dose (ng) for RvE1, Morphine, and NS-398.]
CFA-induced heat hyperalgesia
(Rapid effects: 15-45 min)

- Vehicle
- RvE1, 1 ng
- RvE1, 3 ng
- RvE1, 10 ng
- EPA, 10 μg
- RvD1, 1 ng
- DHA, 10 μg

**PWL (s)**

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>CFA 3 d</th>
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</thead>
<tbody>
<tr>
<td>Value</td>
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</table>

**M.P.E (%)**

<table>
<thead>
<tr>
<th></th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Time after drug injection (CFA, 3 d)
No effect of RvE1 on basal pain
Broad actions of resolvins

(a) Neutrophils
- ↓ Leukotriene B4 functions (RvE1)
- ↓ NF-κB activation (RvE1)
- ↓ Neutrophil infiltration (RvD1/E1)

(b) Macrophages
- ↑ Nonphlogistic phagocytosis of apoptotic neutrophils (RVE1)
- ↓ TNF-α & IL-1β (RvD1/E1)

(c) Microglia

Key:
- Resolvin receptors (GPCRs)

(d) Nerve terminals (skin/muscle)
- ↓ TRPV1 (RvE1)
- ↓ TRPV3 (RvD1)
- ↓ TRPV4 (RvD1)
- ↓ TRPA1 (RvD1)

(e) Neuronal bodies (DRGs)
- ↓ pERK (RvE1)
- ↓ COX-2 (AT-RvD1)
- ↓ NF-κB (AT-RvD1)
- ↓ TNF-α signaling (RvE1)

(f) Presynaptic terminals (Spinal cord)
- ↓ pERK (RvE1)
- ↓ TRPV1 (RvE1)
- ↓ sEPSC (RvE1)
- ↓ Glu release (RvE1)
- ↓ TNF-α signaling (RvE1)

(g) Postsynaptic neurons (Spinal cord)
- ↓ pERK (RvE1)
- ↓ NMDA-R (RvE1)
- ↓ TNF-α signaling (RvE1)

Ji et al., Trends Neurosci, 2011
ChemR23 expression in macrophages and microglia
ChemR23 expression in neurons in the PNS and CNS

(c) ChemR23
(d) Ligation site
(e) Proximal side

Ji et al., Trends Neurosci, 2011
RvE1 blocks capsaicin-induced signaling in mouse DRG neurons

Control

Capsaicin

RvE1 + Capsaicin

300 nM Capsaicin

10 ng/ml RvE1

50 mM KCl

$IC_{50} = 3.2$ ng/ml

Inhibition ratio of capsaicin

0.0

0.2

0.4

0.6

0.8

1.0

1.2

1.4

1.6

1.8

0.1

1

10

ng/ml

340/380

0.4

0.6

0.8

1.0

1.2

1.4

1.6

1.8

0.0

0.2

0.4

0.6

0.8

1.0

1.2

0 sec
**RvE1 blocks TRPV1-mediated spinal synaptic transmission & pain**

*Patch clamp recording*

**Graphs and Data**

- **Control**
- **Capsaicin (100 nM)**
- **RvE1 (1 ng ml) + Capsaicin**

**Bar Graph**

- **Ratio of sEPSC**
  - **Frequency**
  - **Amplitude**

**Duration of nocifensive behavior (s)**

- **Capsaicin (500 ng, i.t)**
- **Capsaicin + RvE1 (10 ng, i.t.)**

*Symbols:* 
- *: p < 0.05
- #: p < 0.01
Resolvins are potent endogenous inhibitors of TRPV1/A1

Table 1. IC\textsubscript{50} for inhibition of TRPV1 and TRPA1 currents by RvD2, RvD1, RvE1, and their fatty acid precursors in DRG neurons

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Molecular weight</th>
<th>TRPV1 IC\textsubscript{50} (nM)</th>
<th>TRPA1 IC\textsubscript{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RvE1</td>
<td>350.4</td>
<td>1.0 ± 0.1</td>
<td>&gt;28.5</td>
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<tr>
<td>RvD1</td>
<td>376.5</td>
<td>&gt;26.6</td>
<td>8.5 ± 0.1</td>
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<tr>
<td>RvD2</td>
<td>376.5</td>
<td>0.1 ± 0.01</td>
<td>2.1 ± 0.5</td>
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<tr>
<td>DHA</td>
<td>328.5</td>
<td>1200.0 ± 20.0</td>
<td>&gt;304,000.0</td>
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<tr>
<td>EPA</td>
<td>302.5</td>
<td>224.0 ± 10.0</td>
<td>&gt;330,578.0</td>
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</tbody>
</table>

TRPV1 and TRPA1 currents were induced by capsaicin (100 nM) and AITC (300 μM), respectively.
Capsaicin and mustard oil-induced pain
Resolvin E1 (RvE1) blocks TRPV1 current in mouse DRG neurons.
ChemR23 is sufficient to mediate RvE1’s effect in HEK 293 cells

TRPV1-expressing HEK cells

Capsaicin (100 nM)  
RvE1 (10 ng/ml)

TRPV1 and ChemR23-coexpressing HEK cells

100 pA  
1 min
RvE1 fails to inhibit TRPV1 signaling in β-arrestin 2 KO mice

WT

β-arrestin 2 KO

Control

RvE1 (10 ng/ml), 15 min
TRPV1 signaling is potentiated in β-arrestin 2 CKO mice

Minutes after capsaicin injection

Number of flicking/licking/biting

Minutes after capsaicin injection
Ion channel research on Primary sensory neurons of human DRGs

Single-cell PCR

<table>
<thead>
<tr>
<th>Sample</th>
<th>TLR7</th>
<th>TRPA1</th>
<th>TRPV1</th>
<th>GAPDH</th>
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<tbody>
<tr>
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<td>neg</td>
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</table>

let-7b (30 µM)

HC030031 (100 µM)
Co-expression of TRPV1 and ChemR23 in human DRG neurons
RvE1 inhibits TRPV1 currents in human DRG neurons

![Image](image.png)

- **RvE1 inhibits TRPV1 currents in human DRG neurons.**

![Graph](graph.png)

- **Graph showing the inhibition of TRPV1 currents by RvE1.**

![Image](image2.png)

- **Image showing a single cell with a scale bar of 25 µm.**

![Image](image3.png)

- **Image showing a microscope view of a cell.**

- **Legend:**
  - **CAP**
  - **RvE1**
  - **Capsaicin. Cap (100 nM)**
  - **RvE1 (100 ng/ml)**

**Quantitative Data:**

- **% of 1st CAP response**
  - **10 ng/ml:** n = 3 / 3
  - **100 ng/ml:** n = 5 / 17
  - **100 ng/ml:** n = 12 / 17
Multiple beneficial effects of resolvins

- Anti-inflammatory actions
- Pro-resolution actions
- Block TNF-α synthesis and signaling
- Modulate synaptic plasticity
- Powerful endogenous inhibitors of TRPV1/A1
- No effects on basal pain
- Unknown side effects

Limitations
- Unstable
- Difficult to measure
- Expensive to produce
Biosynthesis of Protectins from PUFA Precursors

- **EPA**
  - Aspirin:COX-2
  - P450 (microbial)
  - LOX
  - Epoxidation

- **DHA**
  - 17R-Resolvin D Series (Acetylated COX-2)
  - Aspirin:COX-2
  - LOX
  - Lipoxygenase mechanism

- **17S-Resolvin D Series**
  - LOX

- **Protectins & Neuroprotectins**
  - NPD1

- **RvD1**
  - RvE1

- **RvD2**
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