n-3 Docosapentaenoic acid-derived protectin D1 promotes resolution of neuroinflammation and arrests epileptogenesis

Frigerio et al (Brain, Nov. 2018)

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Introduction

Epilepsy

• A group of neurological disorders characterized by epileptic seizures.
• An excessive and disorderly discharge of cerebral nervous tissue on muscles.
• Affects around 65 million people worldwide.
Resolution

• “Clean up” phase after the initial inflammatory response.
• An active biochemical process that involves the interaction of many mediators.

Acute vs. Chronic Inflammation

Fullerton et. al 2016
Pro-resolving Lipid Mediators

- Lipoxins, resolvins, protectins and maresins.
- Key molecules that mediate the active resolution of inflammation in peripheral tissues and in CNS.
- The biological actions mediated by G-protein coupled receptors
  - lipoxin A4 receptor/ formyl peptide receptor 2 (ALX/FPR2)
  - chemerin receptor (ChemR23/ERV1)
Pro-resolving Lipid Mediators

Serhan et al. 2014
# Pro-resolving Lipid Mediators in Disease

<table>
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<tr>
<th>Condition</th>
<th>Mediator</th>
<th>Effect Description</th>
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<tr>
<td>Alzheimer disease</td>
<td>Lipoxins</td>
<td>LXA&lt;sub&gt;4&lt;/sub&gt; decreases NF-κB expression and recruits microglia, promoting clearance of amyloid-β deposits and improving cognition in mouse models</td>
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<td>Protectins</td>
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<td>NPD1 promotes brain cell survival and an anti-apoptotic gene expression programme in human tissue</td>
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<td>Resolvins</td>
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<td>RvD1 stimulates macrophage phagocytosis of amyloid-β &lt;i&gt;in vitro&lt;/i&gt; in PBMCs from patients with Alzheimer disease</td>
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<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Resolvins</td>
<td>RvD1 inhibits IL-6 and TNF production in macrophages derived from post mortem samples</td>
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<tr>
<td>Ischaemic stroke</td>
<td>Resolvins</td>
<td>AT-Resolvin are neuroprotective and limit leukocyte infiltration in mouse stroke models</td>
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Serhan et al. 2014
Materials and Methods

Experimental animals

• Adult male C57/BL6N and NMRI mice
• Free access to food and water, 12h light-dark circle

Mouse model of epilepsy

• An electrode implanted in the dorsal hippocampus
• A cortical surface electrode onto the somatosensory cortex in the contralateral hemisphere
• Two screw electrodes positioned over the nasal sinus and the cerebellum, and used as ground and reference electrodes
Materials and Methods

Detection and quantification of status epilepticus and spontaneous seizures

• Status epilepticus: Spike activity with a frequency >1 Hz intermixed with high amplitude and frequency discharges lasting for at least 5 s, with a frequency of >8 Hz.

• After status epilepticus induction mice were recorded continuously (24/7) until the onset of spontaneous seizures and for 16 days thereafter.

• The end of status epilepticus was defined by the occurrence of interspike intervals longer than 1 s.

• Calculation of total number and total duration of seizures in a 16-day recording period (24/7).
Materials and Methods

Pharmacological treatment with PD1 n-3 DPA-ME

• Mice first exposed to status epilepticus, then assigned randomly to treatment or vehicle (50mM PBS) groups.

• PD1 n-3 DPA-ME, the methylester (ME) pro-drug form of PD1n-3 DPA, which is rapidly converted by cellular esterases to the free acid form.

• PD1n-3 DPA-ME (20 or 200 ng/ml in 50mM PBS) or vehicle was injected intracerebroventricularly (1 ml/site) twice daily for four consecutive days starting 1 h after status epilepticus onset.
Results

Distinct temporal regulation of pro-resolving mediators versus neuroinflammatory mediators during epileptogenesis
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Epileptogenesis alters mouse hippocampal specialized pro-resolving mediator profiles
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PD1 (n-3 DPA) regulates neuroinflammation
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Effect of PD1 (n-3 DPA) on early pathological consequences of status epilepticus
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PD1 (n-3 DPA) reduced ensuing epileptic seizures
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PD1 (n-3 DPA) reduced ensuing epileptic seizures

(B). Note the shorter duration of a typical EEG seizure in a drug-treated mouse versus saline-injected mouse.
Results

PD1 (n-3 DPA) reduced ensuing epileptic seizures

Figure 6  PD1 n-3 DPA reduced spontaneous seizures.

(C) The onset of spontaneous seizures, the number of spontaneous seizures in each day of EEG recording, the average seizure duration and the cumulative time spent in seizures in saline- (n = 12) versus drug-treated mice (n = 9). Data are presented as box-and-whisker plots depicting median, interquartile interval, minimum and maximum (n = number of mice).
Results

PD1 (n-3 DPA) reduced ensuing epileptic seizures

(D) The cumulative number of seizures per day in each experimental group during 16 days after epilepsy onset. *P<0.05; **P<0.01 versus saline injected mice by one-tailed t-test. Friedman’s two-way non-parametric ANOVA was used to detect the treatment effect on number of seizures and duration and their interaction with days. Treatment effect on number of seizures, P<0.01 and interaction with days, P = 0.51; treatment effect on duration of seizures, P<0.01 and interaction with days, P = 0.82.

Figure 6 PD1n-3DPA reduced spontaneous seizures.
Discussion

• Even though early post-injury administration of PD1 (n-3 DPA)-ME significantly reduced neuroinflammation, the number and the duration of spontaneous seizures, it did not reduce neuronal cell loss in the hippocampus.

• Only protectin-D1 administered in follow-up experiments even though resolvin was upregulated as well.

• No difference/increase in inflammatory response after PD1 n-3 DPA treatment in comparison to saline treatment. (P 21)

• Dietary intake of omega-3-polyunsaturated fatty acids → clinical studies with limited results.
Conclusion

• PD1 (n-3 DPA) might be a key player during epileptogenesis.
• Anti-epileptogenic effect of PD1 (n-3 DPA)-ME highlights novel opportunity for drug discovery.
Thank you for listening!