Aspirin-Triggered Neuroprotectin D1 Protects the Penumbra in Focal Cerebral Ischemia in Rats

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Neuroprotectin D1 Inhibits MCA-O Mediated PMN Infiltration, NF-κB Induction, COX-2 Expression and Decreases Infarct Size

MCAo in mouse – 60 min
NPD1 infused into 3rd ventricle
(400 ng/2 days)
Histology – 48 h

Endogenous NPD1

PMN infiltration

Myeloperoxidase

Contralateral  Ipsilateral (MCA-O)

Vehicle

Neuroprotectin D1

NFκB induction
•  COX-2 expression
•  Stroke size

Marcheselli, et.al., JBC (2003)
DHA Potentiates NPD1 Synthesis in the Penumbra 3 Days After MCAo

Sprague Dawley rats
MCAo - 2 hours
DHA (5 mg/kg) or Saline, 3h after onset of ischemia
Lipidomic analysis on day 3

Neuroprotectin D1 (NPD1), the DHA-derived lipid mediator, and 17-HDHA (a marker of 17-H(p)DHA, the short lived NPD1 precursor) were isolated from the penumbra using mass spectrometer (LC-UV-MS/MS)

P: penumbra
C: core
Neuronal COX-2 Expression is Enhanced During Synaptic Network Disinhibition

COX-2 (yellow), MAP2 (red), and DNA (blue) imaged as a Z-stack by laser confocal microscopy. Data rendered in 3D.

Synaptic and Extrasynaptic NMDA Receptors Differentially Modulate Neuronal Cyclooxygenase-2 Function, Lipid Peroxidation, and Neuroprotection
D. Stark and N. Bazan
The Journal of Neuroscience: 13710-13..2011
OBJECTIVE:

- **Does docosahexaenoic acid plus aspirin elicits the synthesis of a novel NPD1 in the MCA-O penumbra?**
- **If so, is the new mediator endowed with bioactivity?**

- DHA is the precursor of **neuroprotectin D1 (NPD1)**, potent modulator of neuroinflammation and neuroprotective.

- **Aspirin** (nonsteroidal anti-inflammatory Drugs, NSAIDs).
- Irreversibly inhibitor of COX-1 by acetylation of a lysine in the active site.
Novel Proresolving Aspirin-Triggered DHA Pathway

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SUMMARY

Endogenous mechanisms in the resolution of acute inflammation are of interest because excessive inflammation underlies many pathologic abnormalities. We report an aspirin-triggered DHA metabolome that biosynthesizes a potent product in inflammatory exudates and human leukocytes, namely aspirin-triggered Neuroprotectin D1/Protectin D1 [AT-(NPD1/PD1)]. The complete stereochemistry of AT-(NPD1/PD1) whereas resolvins and protectins promote and stimulate active resolution (Bazan et al., 2010; Serhan et al., 2002; Serhan and Savill, 2005). In excess, prostaglandins and leukotrienes are proinflammatory (Samuelsson, 1983).

Unlike many of the current anti-inflammatory agents, which delay complete resolution and are considered toxic to this vital process (i.e., resolution toxic) (Gilroy et al., 1999; Schwab et al., 2007), aspirin is unique in that it jump-starts resolution by novel previously unrecognized mechanisms that involve the biosynthesis of aspirin-triggered (AT) lipid mediators (Gilroy and Perretti, 2005; Serhan, 2007). For example, aspirin-triggered
Characterization of Aspirin Triggered-Neuroprotectin D1 (AT-NPD1) and Evidence for its Biosynthesis after MCA-o upon DHA+Aspirin (iv)

N. Bazan, et al, (submitted)
LC MS/MS Lipidomic Study

DHA+Aspirin Induces Neurological and Histopathological Protection with Concomitant Synthesis of AT-NPD1

- AT-NPD1 synthesis increased by DHA+Aspirin.
- NPD1 and 17HDHA increased by DHA,
- PGE$_2$ reduced by Aspirin and DHA+Aspirin.
**Animals:** Male Sprague Dawley rats (285-360 g)

**Anesthesia:** Isoflurane/nitrous oxide/oxygen

**Physiological Monitoring:** Rectal and cranial temperatures, arterial blood gases (pO$_2$, pCO$_2$) and pH, plasma glucose, hematocrit

Middle cerebral artery occlusion - 2 hours

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**Experimental Protocol**

- **MCAo – 2h**
  - Treatment
  - Behavioral testing

**Ex vivo MRI, Immunohistochemistry**

- AT-NPD1 (Sodium salt), 333 µg/kg, IV
- AT-NPD1 (Methyl Ester), 333 µg/kg, IV
- Saline at 3h after onset MCAo, IV
Both AT-NPD1 treatments improve total neurological score

Belayev, et al, 2012 (under review)
Both AT-NPD1 treatments reduce cortical lesion
Both AT-NPD1 increase SMI-71 positive vessels and GFAP positive astrocytes and decrease ED-1 microglia/microphages cell count.
Immunohistochemistry

Computer-generated MosaiX processed images of SMI-71 (positive vessels), GFAP (positive astrocytes), ED-1 (positive microglia/microphages) and GFAP/ED-1 double staining
Histopathology (day 7)

Both AT-NPD1 reduce cortical, subcortical and total infarct areas and volumes.
White Matter

AT-NPD1-ME enhances white matter reorganization
3D Volumes Were Computed From T2WI on day 7

Both AT-NPD1 treatments reduce total lesion
T2-Weighted Imaging: Brain Edema and Water Mobility

AT-NPD1 treatment reduces brain edema and improves water mobility.
Both AT-NPD1 treatments reduce subcortical lesion volumes.
Both AT-NPD1 treatments reduce total lesion volumes.
Biosynthesis and Mechanism of Action of Neuroprotectin D1 (NPD1) and of Aspirin-Triggered Neuroprotectin D1 (AT-NPD1)

**PLA$_2$**

Free DHA

- **Acetylated COX-2**
- **15-LO-1**

Neuroprotectin D1 (NPD1)

Neurotrophins (e.g. PEDF, BDNF, CNTF, LIF, NT3)

- **Neurotrophins**
  - 15-LO-1
  - Neuroprotectin D1 (NPD1)

Docosanoids

- **Aspirin-triggered Neuroprotectin D1 (AT-NPD1)**
- **15-LO-1**

Potent Neuroprotective Bioactivity in MCA-O

Bioactivity

- **PMN Infiltration**
- **Pro-inflammatory Gene expression**
  - COX-2,CEX1,B-94 Polymorphonuclear Leukocytes infiltration and Activation (PMNs)
- **Pro-Apoptotic Bcl-2 Proteins**
  - PP2A-BCLxl-Bax
- **Anti-Apoptotic Bcl-2 Proteins**

PPRgamma

Akt-1 (Akt/PKB) and m-Tor

Cornea nerve regeneration

- PPRgamma
- Akt-1 (Akt/PKB) and m-Tor
- Cornea nerve regeneration
CONCLUSIONS

• AT-NPD1 and AT-NPD1-methyl-ester *improved neurological deficit* when treatment is administered up to 3 h after onset of ischemia.

• AT-NPD1 and AT-NPD1-methyl-ester *reduced T2 WI total, cortical and subcortical lesion areas and volumes*.

• AT-NPD1-methyl-ester appear to be more beneficial on neurological deficits and lesion volume outcome compared to AT-NPD1 in sodium salt formulation.
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