Investigating Neuroprotective Efficacy of Lipid Mediators Neuroprotectin D1 (NPD1) and Resolvin D1 (RvD1) on Behavioral Outcomes and Ischemic Penumbra

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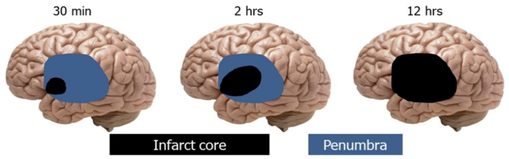
Introduction

Ischemic Stroke

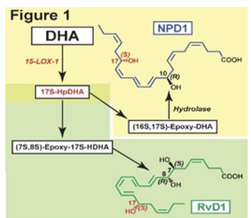
- In the United States, stroke is the sixth leading cause of death, and a major cause of long-term disability.
- It is estimated that the average economic burden of stroke-related costs is over $35 billion annually.
- Over 87% of strokes are ischemic strokes which are caused by a thrombus blocking blood flow through one of the cerebral arteries. This results in an ischemic core of permanently damaged tissue with a surrounding penumbra of “at-risk” tissue.
- The ischemic penumbra has the potential to be salvaged if there is re-perfusion of tissue within a certain window of time; however, there is currently no treatment options that reduce the acute immune and inflammatory responses to stroke.



Docosanoid Lipid mediators

- Docosanoid lipid mediators are essential omega-3 fatty acid derivatives that have anti-inflammatory properties.
- These lipid mediators are stored as esterified phospholipids within cell membranes that are cleaved into Arachadonic acid and Docosahexaenoic acid (DHA) upon cell injury.
- In the brain, DHA is released following stroke, and it is converted into Neuroprotectin D1 (NPD1) via 15-lipoxygenase and Resolvin D1 (RvD1) via 15- and 5-lipoxygenase.
- It has been proven that DHA, NPD1, and RvD1 are neuroprotective after an experimental ischemic stroke. Along with helping to reduce inflammation, these compounds promote cell survival, improve neurologic outcomes, and reduce infarct volumes.

Fig. 1: Synthesis of NDP1 and RvD1
DHA is converted to both Neuroprotectin D1 (NPD1) and Resolvin D1 (RvD1) via 15- and 5-lipoxygenase, respectively



Aim: In this study, we tested the neuroprotective combinatory effects of IV administered NPD1 and RvD1 on behavioral outcomes and the ischemic penumbra 7 or 14 days after experimental stroke using a well-established rat model of middle cerebral artery occlusion (MCAo).

Methods

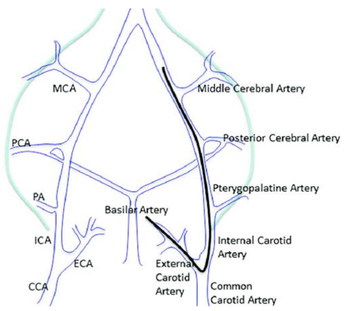


Fig. 2: Right MCAo Model
An illustration of an intraluminal suture occluding the right Middle Cerebral Artery (MCA) upon insertion

Middle Cerebral Artery Occlusion (MCAo) stroke model

Male Sprague-Dawley rats were anesthetized and received 2h of right MCAo by intraluminal suture. After 2h, the suture was removed to allow for the restoration of blood flow. During surgery, the following physiologic parameters were measured: temperature (rectal and cranial), arterial blood pressure, arterial blood gases (pO_2, pCO_2), pH, blood glucose, and hematocrit.

Methods

Behavioral testing

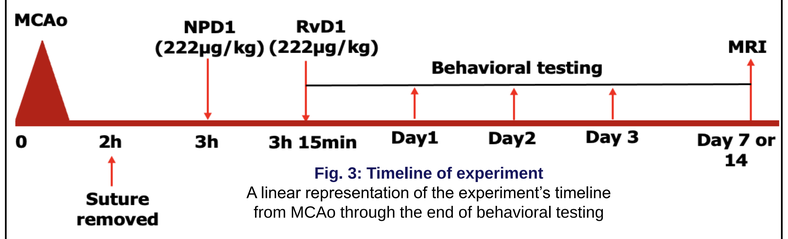
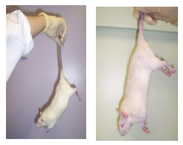


Fig. 3: Timeline of experiment
A linear representation of the experiment’s timeline from MCAo through the end of behavioral testing

(a) Postural reflex test



(b) Forelimb Placing Test

(a) Postural reflex test is used to examine upper body posture while the animal is suspended by the tail. (b) Forelimb placing test is used to examine sensorimotor integration in forelimb placing responses to visual (forward and sideways), tactile (lateral and dorsal), and proprioceptive stimuli. Grading Scale is used on each individual test is given a score of 0 to 2 and summed together to give a total score between 0 (normal) to 12 (maximum deficit)

Treatment Groups

Animals were randomly assigned to four different treatment groups: NPD1 (222µg/kg) + RvD1 (222µg/kg) 7-day or 14-day survival, or vehicle (saline) 7-day or 14-day survival (n=6-11 per group). At 3h after MCAo, vehicle or NPD1 was administered followed by RvD1 15 minutes after NPD1. Composite behavioral (neurologic) tests done on days 1, 2, 3, 7, or 14 which was followed by perfusion on days 7 or 14 and MRI.

Group A: Saline (7-day) n=11
Group B: NPD1 + RvD1 (7-day) n=6
Group C: Saline (14-day) n=11
Group D: NPD1 + RvD1 (14-day) n=6

Results-Total Neurologic Score

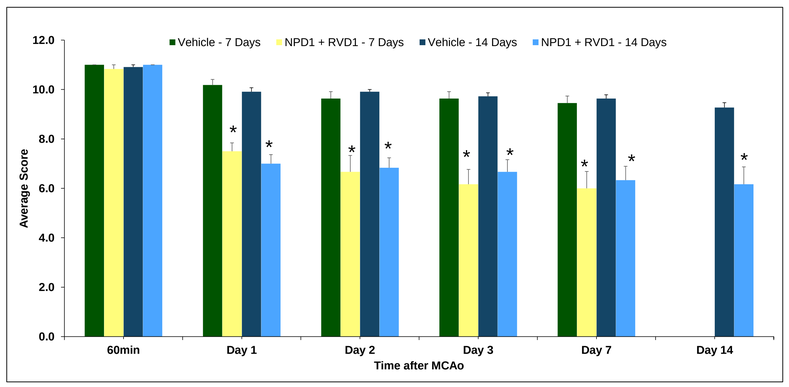


Fig. 4: NPD1 + RvD1 improve total neurologic behavioral score after MCAo
Total Neurologic score is measured on a scale of 0 (normal) to 12 (maximal deficit). These measurements were taken during MCAo (60min) and days 1, 2, 3, 7, and 14 after treatment. At 60 min of MCAo, animals had a score of 10 or 11. Total neurologic (behvaioral) score improved after treatment for both the 7-day and 14-day survival period compared to vehicle group. (*P <0.05 vs vehicle group)

Results-Individual Neurologic Scores

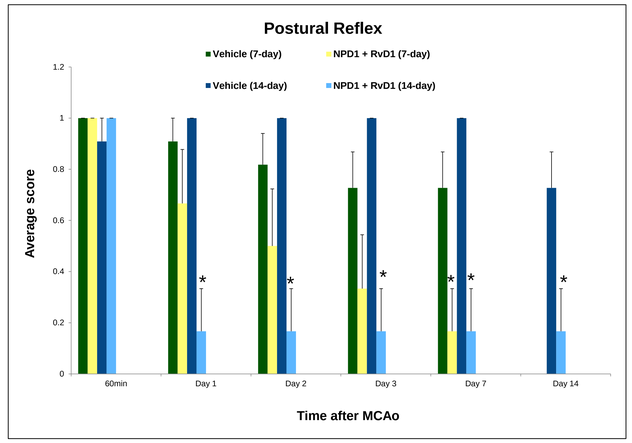


Fig. 5: NPD1 + RvD1 improve Postural reflex neurologic score
Postural reflex is measured on a scale of 0 (normal) to 2 (maximal deficit). These measurements were taken during MCAo (60min) and days 1, 2, 3, 7, and 14 after treatment. Postural reflex score improved after treatment for both the 7-day and 14-day survival period compared to vehicle group. (*P <0.05 vs vehicle group)

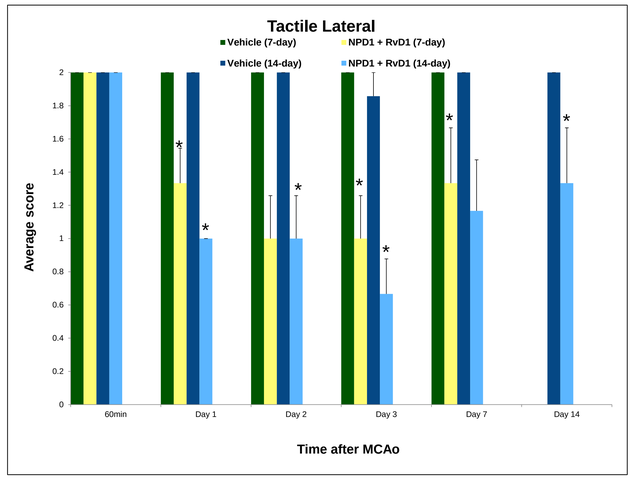


Fig. 6: NPD1 + RvD1 improve tactile lateral neurologic score
Tactile lateral is measured on a scale of 0 (normal) to 2 (maximal deficit). These measurements were taken during MCAo (60min) and days 1, 2, 3, 7, and 14 after treatment. Tactile lateral score improved after treatment for both the 7-day and 14-day survival period compared to vehicle group. (*P <0.05 vs vehicle group)

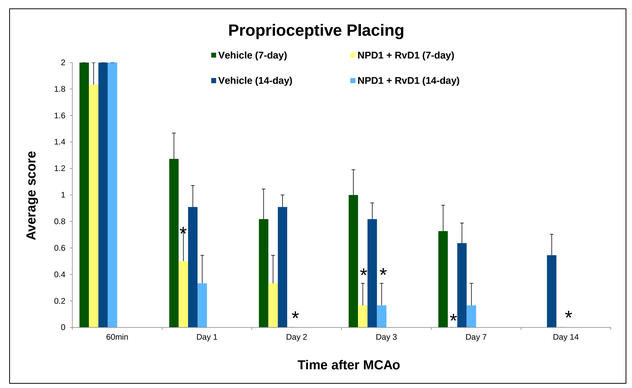


Fig. 7: NPD1 + RvD1 improve proprioceptive placing neurologic score
Proprioceptive placing is measured on a scale of 0 (normal) to 2 (maximal deficit).These measurements were taken during MCAo (60min) and days 1, 2, 3, 7, and 14 after treatment. Proprioceptive placing score improved after treatment for both the 7-day and 14-day survival period compared to vehicle group. (*P <0.05 vs vehicle group)

Conclusions

We concluded that the intravenous administration of NPD1+RvD1 in combination after stroke both 7-days and 14-days after MCAo improves behavioral outcomes when compared to the vehicle groups.

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