

Novel Docosanoids Elicit Neuroprotection After Experimental Stroke in Female Rats

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Introduction

Stroke is the fifth leading cause of death and long-term health complications. Tissue plasminogen activator, most commonly known as tPA, is a potent blood thinner used for emergency stroke treatment and only an approved drug that should be administered within 4.5h of stroke onset, but still only 5-8% of patients qualify for this therapy. Sex and gonadal hormone exposure have considerable independent impact on stroke outcome. In the United States, 1 in 5 women between the ages of 55 and 75 will have a stroke. Women accounted for 57.1% of stroke deaths in 2019, with stroke accounting for 6.2% of all female deaths, while comprising 4.4% of all male deaths. Previously, Dr. Alkayed reported stroke study in female rats (*Alkayed et al., 1998, Stroke*). They conducted 2 hours of MCA occlusion and histopathology at 24 hours. The results were that female rats have smaller infarcts compared to male rats. Females maintain a higher CBF than males at the end of vascular occlusion. They concluded that endogenous estrogen improves stroke outcome (*Alkayed et al., 1998, Stroke, 29, 159-166*).

Neuroprotectin D1 (NPD1; 10R, 17S-dihydroxy-4Z, 7Z, 11E, 15Z, 19Z-docosahexaenoic acid) is a potent lipid mediator synthesized on demand at the onset of uncompensated oxidative stress to sustain homeostasis. It is a modulator of inflammation resolution that promotes cell survival and neurogenesis, inhibits leukocyte infiltration and pro-inflammatory gene expression, attenuates edema formation, and reduces stroke volume after MCAo. It inhibits oxidative stress-induced caspase 3 activation and protects cells from oxidative stress-induced apoptosis. It also upregulates the anti-apoptotic proteins such as Bcl-2 and Bcl-xL and decreases pro-apoptotic Bax and Bad expression. This was all discovered and described by *Bazan (2005)*.

Resolvin D1 (RvD1; 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) is an important endogenous mediator that suppresses the inflammatory response. It decreases inflammatory cell migration in inflamed tissues, promotes phagocytosis of apoptotic cells, and reduces the expression of inflammatory factors. RvD1 was never studied in the MCAo stroke model. Still, it was demonstrated that DHA-derived D-series resolvins reduced inflammation in different disease models, such as kidney injury and cardiovascular and autoimmune disorders.

In this study, the effects of Neuroprotectin D1 and RvD1 are investigated in female Sprague Dawley rats, to see if similar neuroprotective properties can be observed in comparison to a previous study in male Sprague Dawley rats (*Reid et al., 2023, Cellular and Molecular Neurobiology*)

Materials and Methods

Animals:

All animals used were female Sprague-Dawley rats weighing 280-300 grams. 31 female Sprague Dawley rats were used in this experiment.

Surgical Preparation:

Female Sprague-Dawley rats (280-300g) are fasted overnight prior to the day of surgery but allowed free access to water. The rats are anesthetized with isoflurane (3-1%), Nitrous Oxide (70%), and Oxygen (30%). Then orally intubated and mechanically ventilated under 1% isoflurane and the same ratio of gasses. Cranial and rectal probes were inserted and in place throughout surgery to monitor temperature. Catheters were inserted into the right femoral artery and vein for blood sampling and drug infusion. Arterial blood gases, plasma glucose, arterial blood pressure, and heart rate were monitored and analyzed before and after the middle cerebral artery occlusion.

Middle Cerebral Artery Occlusion:

To induce a stroke, a nylon suture coated with poly-L-lysine is inserted in the external carotid artery and carefully maneuvered so that it eventually reaches the middle cerebral artery to block MCA (*Belayev et al., 1996*). Occlusion was confirmed by performing a neurobehavioral test sixty minutes after MCA occlusion (MCAo) on a scale of 0-12 (0=no deficit, 12=maximal deficit). Only those with a high-grade deficit (>10) were used. The suture is introduced for 2 hours and then removed.

Treatments:

There were four treatment groups: **Vehicle**, **NPD1**, **RvD1**, and **NPD1+RvD1 (combinatory)**. The **vehicle** (IV, 0.9% saline, 1 mL/kg + 10% ethanol 1 mL/kg, n=9) or **NPD1** (IV, 222µg/kg, n=10) was administered at 3 hours after MCAo. **RvD1** (IV, 222µg/kg, n=5) was administered 15 minutes after. The **combinatory treatment group** (n=7) consisted of the same doses and concentrations as the previous NPD1 & RvD1 groups.

Neurological Testing:

Behavioral testing is being used as a clinically relevant outcome measure and to identify animals that should be excluded. There are six behavioral tests all with scores from 0 to 2. Total behavioral scores range from 0 to 12, with the lowest score (0) being the normal score, and the highest score (12) representing maximal deficit. All animals were tested for behavior at one hour before treatment to ensure a presence of MCAo.

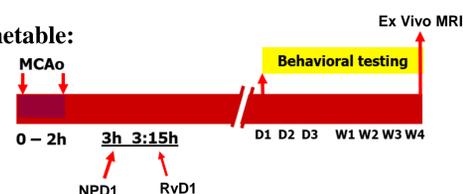
Table 1:

	Normal	Maximal
Postural Reflex	0	2
PLACING TEST		
Visual Placing		
<i>Forward</i>	0	2
<i>Sideways</i>	0	2
Tactile Placing		
<i>Dorsal Surface of Paw</i>	0	2
<i>Lateral Surface of Paw</i>	0	2
Proprioceptive Placing	0	2
TOTAL score for Placing	0	12

Statistical Analysis

Values are presented as means±SEM. Two-tailed Student's t-tests were used for two-group comparisons. A value of $p < 0.05$ was considered statistically significant.

Experimental Timetable:



Results

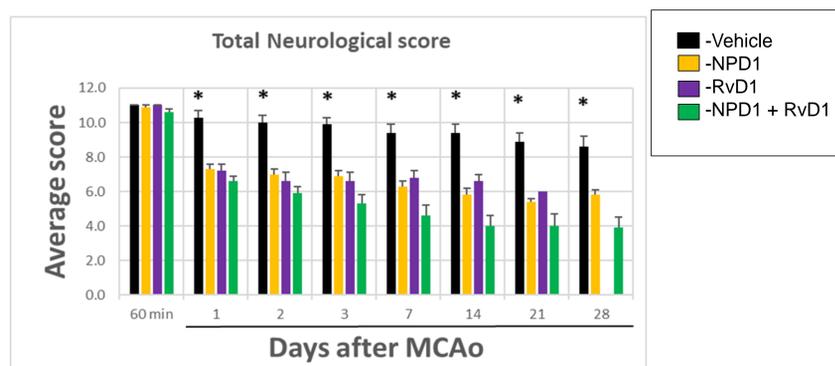


Figure 1: Total Neurological Score:

NPD1, RvD1 and NPD1 + RvD1 improved total neurological score during 28 days survival period compared to vehicle group. Total neurologic score (normal=0, maximal deficit=12) during 60 min of MCAo and days 1, 2, and 3, and weeks 1, 2, 3, and 4 after MCAo. All treatments improved total neurological scores compared to the vehicle group. Data are mean±SEM, n=5-10 per group. * $p < 0.05$, student t-test.

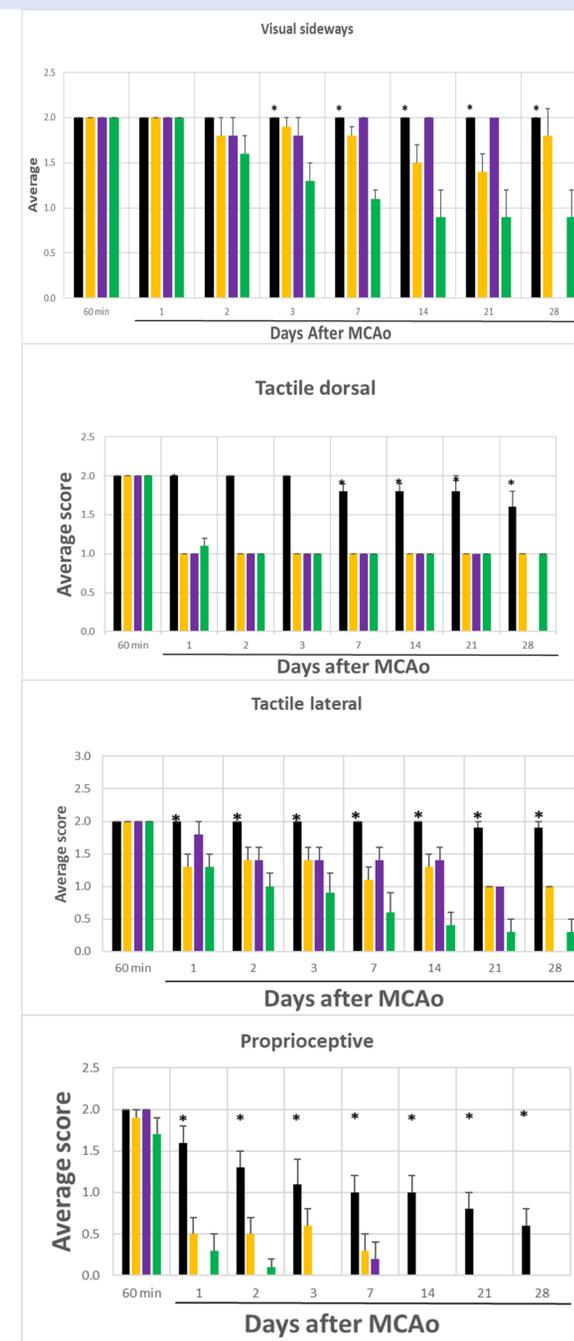
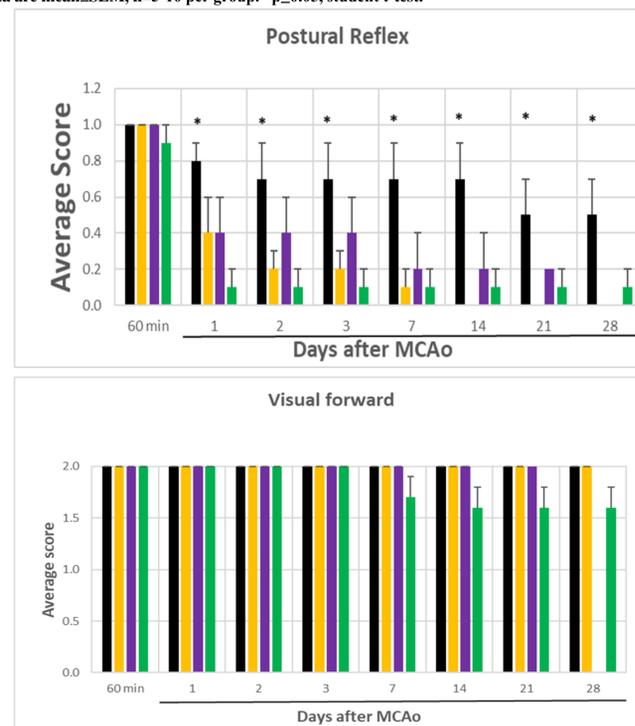


Figure 2: Time Course Recovery Behavioral Functions Individual Scores

NPD1, RvD1, and NPD1 + RvD1 improved individual behavioral scores for postural reflex, visual forward, visual sideways, tactile dorsal, tactile lateral, and proprioceptive scores during 28 days survival period compared to vehicle group. Individual behavioral scores (normal=0, maximal deficit=2) during 60 min of MCAo and days 1, 2, and 3, and weeks 1, 2, 3, and 4 after MCAo. All treatments improved individual behavioral scores compared to the vehicle group. Data are mean±SEM, n=5-10 per group. * $p < 0.05$, student t-test.

Conclusions

- **NPD1 and RvD1** treatments when administered alone showed significant behavioral improvement compared to the vehicle in female rats.
- **Combinatorial Therapy** showed the largest behavioral improvement compared to its vehicle.
- On days 2 and 3, **combinatorial therapy** produced improvements in neurobehavioral testing compared to NPD1.
- On days 7 and 14, **combinatorial therapy** produced improvements in neurobehavioral testing compared to RvD1.
- There were no adverse side effects observed with any of the treatments tested.
- This combinatorial treatment would contribute to open potential therapeutic avenues for ischemic stroke.

References

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