Long-lasting Neuroprotection by LAU-0901 and Neuroprotectin D1 Following Experimental Ischemic Stroke in Rats

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Introduction

In the United States there are nearly 800,000 cases of stroke each year (CDC, 2022). It is the fifth greatest cause of death in the U.S., responsible for approximately 1 in 6 deaths annually (Virani et al., 2021). Approximately 85% of all strokes are ischemic (Guzik and Bushnell, 2017). Patients who have had an acute ischemic attack are extremely likely to suffer from debilitating disabilities. Ischemic stroke produces lesions in the areas of the brain that necrose due to an insufficient supply of blood, this area is known as the ischemic core. Damaged, but salvageable tissue that surrounds the core is the penumbra.

Neuroprotectin D1 (NPD1; 10R, 17S-dihydroxy-4Z, 7Z, 11E, 15Z, 19Z-docosahexaenoic acid) is an endogenous lipid mediator that is produced in the brain on demand to protect against oxidative stress. It does so by inhibiting polymorphonuclear (PMN) infiltration, reduces stroke-mediated damage, and downregulates ischemia-related gene expression (Belayev et al., 2018). NPD1 is produced as needed, primarily in response to oxidative stress and/or neurotrophin activation in the brain and retina from docosahexaenoic acid [DHA; 4 22:6 (n-3)], an omega-3 essential fatty acid that facilitates and influences an array of neurological functions (Niemoller and Bazan, 2010). The enzyme responsible for NPD1 production is 15-lipoxygenase-1. NPD1 is frequently one of the first lines of defense in response to a disruption of homeostasis as a result of neuronal injury. It has been shown experimentally that it protects cells in a number of neurodegenerative diseases such as Huntington's disease, Alzheimer's and retinitis pigmentosa (Bazan 2012). NPD1 also displays significant protective capabilities in response to much more acute neuronal injury such as stroke and traumatic brain injury (Asatryan and Bazan, 2017).

LAU-0901 (LAU; 2,4,6-trimethyl-1, 4-dihydro-pyridine-3, 5-dicarboxylic acid) is a potent antagonist of platelet activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-gycero-3-phosphocholine) receptors (Belayev et al., 2008). PAF is a phospholipid mediator that greatly mediates inflammation throughout the body, including the brain (Ashraf and Nookala, 2022). PAF accumulates in the brain during cerebral ischemic injury, specifically upon reperfusion. The accumulation of PAF in this context greatly increases inflammation and creates a cytotoxic environment (Belayev et al., 2012). Both are extremely harmful stressors in the brain by inhibiting normal cellular function and promoting apoptotic pathways (Puyal, Ginet, and Clarke, 2013). However, Belayev et al. showed that LAU is extremely effective at downregulating the accumulation of PAF in ischemic stroke and thus reducing brain tissue damage (2012).

The potent neuroprotective qualities that both NPD1 and LAU-0901 posses in experimental ischemic stroke inspires the idea of a synergistic treatment of both molecules in treating ischemic stroke.

Materials and Methods

Animals:
All animals used were male Sprague-Dawley rats weighing 240-370 grams.

Surgical Preparation:
Animals were fasted overnight prior to surgery but allowed free access to water. All rats were anesthetized by inhalation of 3% isoflurane in a 70% NO and 30% O_2 mixture. 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 heartrate were monitored and analyzed before and after the middle cerebral artery occlusion.

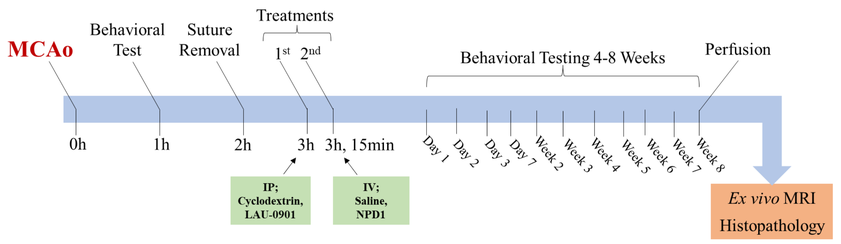
Middle Cerebral Artery Occlusion:
The right middle cerebral artery (MCA) was occluded by 4cm of a poly-L-lysine coated 3-0 monofilament nylon suture. The filament was inserted through the external carotid artery (ECA) and into the internal carotid artery (ICA) to the MCA as described by Longa et al. (1989). 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. After two hours of MCAo, the suture is removed to simulate reperfusion of the ischemic area.

Treatments:
There were four different possible treatment groups: Control, LAU-0901, NPD1, and Combination. The control group (n=21) received cyclodextrin (IP, 45%, 1mL/kg) for the first treatment and saline (IV, 0.9%, 1mL/kg) for the second. LAU-0901 (IP, 60mg/kg, n=23) was dissolved in cyclodextrin and administered as the first treatment. NPD1 (IV, 222µg/kg, n=14) was dissolved in saline and given as the second treatment. The combinatory treatment (n=14) consisted of the previous doses of LAU-0901 and NPD1.

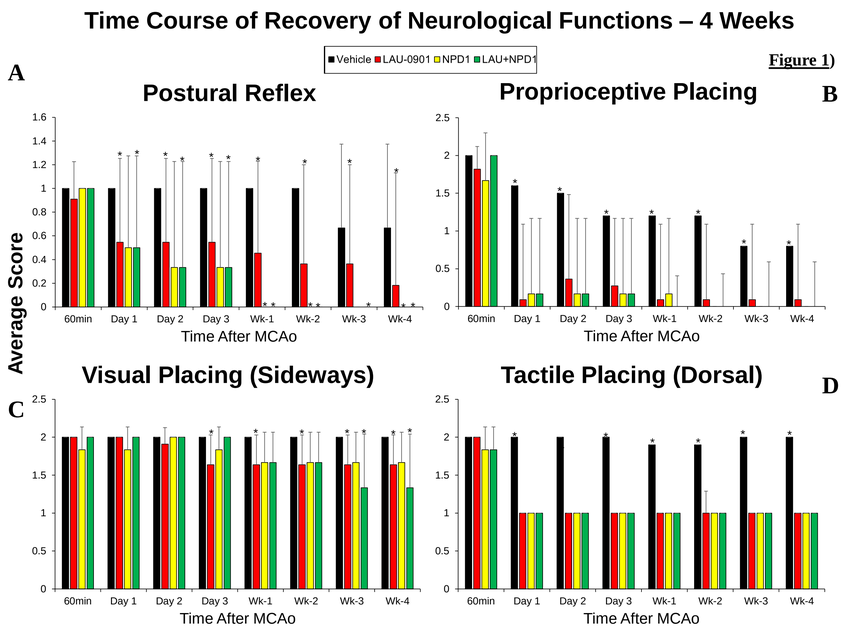
Neurological Testing:
All animals were subjected to behavioral testing following MCAo to measure neurological deficits after ischemic insult. Two tests were conducted: a postural reflex test that examines upper body posture while suspended by the tail, developed by Bederson et al. (1986), and a placing test of the forelimbs that observes sensorimotor function in response to visual, tactile, and proprioceptive stimuli, developed by De Ryck et al. (1989). These tests were performed at multiple intervals after MCAo: 60 minutes, days 1-3, and up to 4 or 8 weeks depending on the experimental group of the rat.

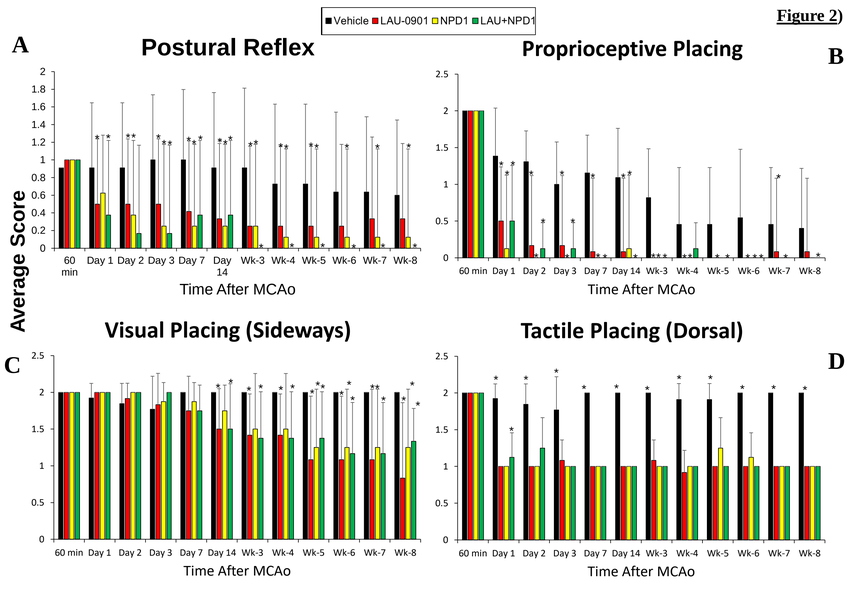
Magnetic Resonance Imaging and Histopathology
Following perfusion with 4% paraformaldehyde on either Week 4 or 8, the brains were removed to be sent for magnetic resonance imaging (MRI) and histopathology in order to analyze the size of lesions in the contralateral and ipsilateral hemispheres of ischemic insult. This portion of the study is ongoing.

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.



Results





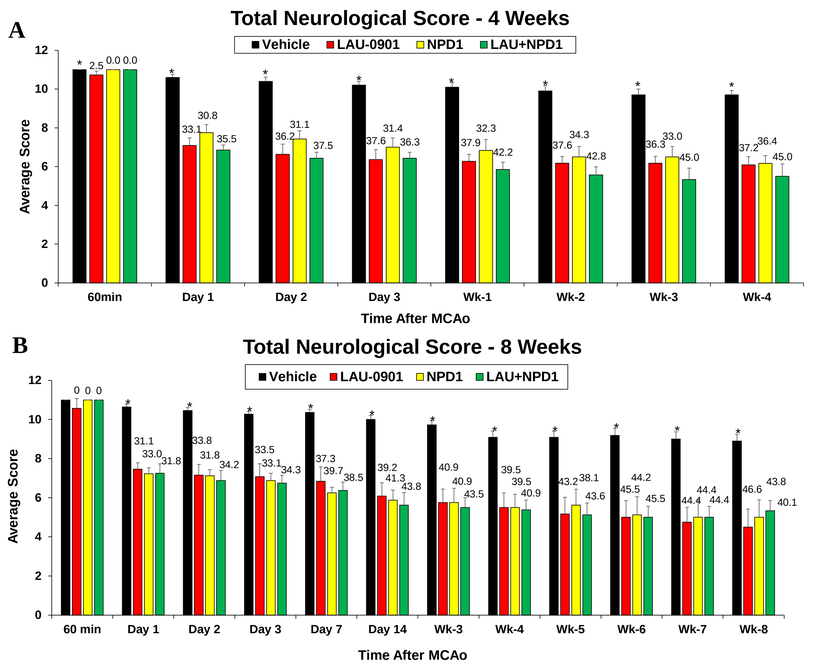


Figure 3) The total neurobehavioral scores of animals observed of the Vehicle, LAU-0901, NPD1, and LAU+NPD1 groups. **(A)** Represents the neurological scores of rats observed for 4 weeks. All three treatment groups significantly improved neurobehavioral scores compared to the control group on Day 1 and throughout the remainder of observation ($p<0.05$). However, differences between the three treatment groups were not significant at any point. **(B)** The neurological scores of the animals observed over 8 weeks. Following the day of MCAo, all treatment groups demonstrated significant recovery compared to the vehicle group ($p<0.05$). Differences between treatment groups are insignificant.

Conclusions

Treatments

- LAU-0901, NPD1, and the combination treatment all demonstrated extremely significant recovery compared to the control.
- No significant difference between neuroprotective treatment groups in recovery over 4 or 8 weeks.
- Ultimately, the combinatory treatment does not demonstrate to have significantly better or worse neuroprotective properties than either treatment alone.

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