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“Expression of pro-homeostatic astrocytic genes in ipsilesional ischemic core and penumbra in rats after ischemic stroke: Neuroprotection by NPD1 plus RvD1.”

Background: Stroke is the second leading cause of death worldwide, causing one in four people over the age of 25 to have a stroke during their lifetime. 62.4% of all strokes are ischemic strokes and happen when a blockage cuts off the blood supply to an area of the brain. The interrupted blood flow causes brain cells to die within minutes. Following cell death, a secondary inflammatory response occurs, which can cause further damage. Currently, there is no effective neuroprotective therapy available to mitigate ischemic stroke. It has been previously shown that Omega-3 polyunsaturated fatty acids (PUFAs) have positively impacted cerebrovascular diseases, including carotid stenosis, vertebral and intracranial stenosis, aneurysms, and vascular malformations. By limiting inflammatory responses and promoting specific mechanisms to return to homeostasis, certain endogenous protective docosanoids, Neuroprotection D1 (NPD1) and Resolvin D1 (RvD1), have proven to help protect against cerebral ischemia in a rat model of middle cerebral occlusion (MCAo) (Reid et al, PMID: 37270727). **Objective:** We aim to understand further the mediative and protective affects NPD1 and RvD1 have on the brain through analysis of neuroprotective and anti-inflammatory gene expressions in the ischemic core and penumbra.

Methods: Ischemic stroke was induced in rats using the transient middle cerebral artery occlusion (MCAo) for 2h (experiments conducted recently in the lab). 1h after, rats were re-anesthetized and treated with either vehicle (0.9% saline) or NPD1 + RvD1. 24h later, the rat was euthanized, and the brain was flash-frozen in methyl butane pre-chilled with dry ice. Then, brain sections were embedded with an Optimal Cutting Temperature (OCT) medium in a cryomold. The brain was then cryosectioned into 10-micron slices and placed onto positively charged Superfrostplus slides. The slides were then fixed with neutral buffered formalin, followed by alcohol dehydration, and subjected to multiple RNAscope probes (for multiplexing). We then imaged the slides and analyzed the data in Olympus Flouveiw, and Cellsens software followed by unbiased image analysis using FIJI ImageJ.

Results: We selected some astrocytic genes – pan-reactive (*Cxcl10*, and *Lcn2*), A1 neurotoxic (*Amigo2*), and A2 neuroprotective (*Thbs1*, and *Tm4sf1*) and looked at the expression of these genes in ipsilesional ischemic core and penumbra and compared between rats subjected to stroke treated with vehicle and rats treated with neuroprotective lipid mediators – NPD1 + RvD1.

Conclusions: Our single molecular RNA fluorescence *in situ* hybridization (FISH) results validated results obtained in the lab using bulk RNA extraction followed by high density quantitative PCR after subjecting rats to transient MCAo, followed by treatment with docosanoids – NPD1 + RvD1. This is the first time that selected probes were demonstrated to work for the groups of selected genes. These observations uncover spatially distributed specific genes being expressed during neuroprotection elicited by the lipid mediators and will be important to guide potential therapeutics.