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A Preliminary Report on the Role of Lipoxin A4 in Reinstating the Blood-Brain Barrier Integrity in a Rodent Model of Acute Inflammation with Impaired Cerebrovasculature

Minjal Patel
Rowan University

Shruti Varshney
Rowan University

Ananya Nethikunta
Rowan University

George G. Godsey
Rowan University

Mary C. Kosciuk
Rowan University

See next page for additional authors

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Minjal Patel, OMS-II[1], Shruti Varshney, OMS-II[1], Ananya Nethikuntha[1], George G. Godsey[2], Mary C. Kosciuk[2], Ana Rodriguez[3], Bernd W. Spur[3], Kingsley Yin[3], Randel L. Swanson[4-6], Venkat Venkataraman [3, 7], Robert G. Nagele[1-2,8], and Nimish K. Acharya[1-4,8]

1. Rowan-Virtua School of Osteopathic Medicine (**Rowan-Virtua SOM**), Stratford, NJ, USA. 2. Department of Geriatrics and Gerontology New Jersey Institute for Successful Aging (NJISA), Rowan-Virtua SOM, Stratford, NJ, USA. 3. Department of Cell Biology & Neuroscience, Rowan-Virtua SOM, Stratford, NJ, USA. 4. Center for Neurotrauma, Neurodegeneration and Restoration, Corporal Michael J. Crescenz VA Medical Center, Philadelphia, USA. 5. Department of Physical Medicine and Rehabilitation, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA. 6. Rehab Medicine Service, Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA, 19104, USA. 7. Department of Academic Affairs, Rowan-Virtua SOM, Stratford, NJ, USA. 8. Rowan-Virtua School of Translational Biomedical Engineering & Sciences, Rowan University, Glassboro, NJ, USA.

Abstract

Background: The blood-brain barrier (**BBB**) is responsible for maintaining brain homeostasis and ultimately proper neuronal function. Disruption of the BBB, leading to increased BBB permeability, has been reported in several neurodegenerative diseases, including Alzheimer's disease (**AD**) and traumatic brain injury (**TBI**). Lipoxins (**LXs**) are a class of arachidonate-derived eicosanoids, which are a class of specialized pro-resolving lipid mediators (**SPMs**). SPMs are known to inhibit immune response through inhibition of cellular infiltration, downregulation of pro-inflammatory mediators and upregulation of anti-inflammatory mediators. Hence, LXs are recognized as "breaking signals" in the inflammatory process. One form of LXs, Lipoxin A4 (**LXA4**), has been found to decreased production of proinflammatory mediators, inhibit neutrophils chemotaxis and infiltration to the site of injury, and promote the phagocytic clearance of debris by macrophages. Therefore, LXA4 serves a critical role in resolution of inflammatory process by regulating the activation of monocytes and modulating the generation of reactive oxygen species (ROS).

Hypothesis: LXA4 treatment reinstates the BBB integrity in a rodent model of acute BBB breakdown and inflammation.

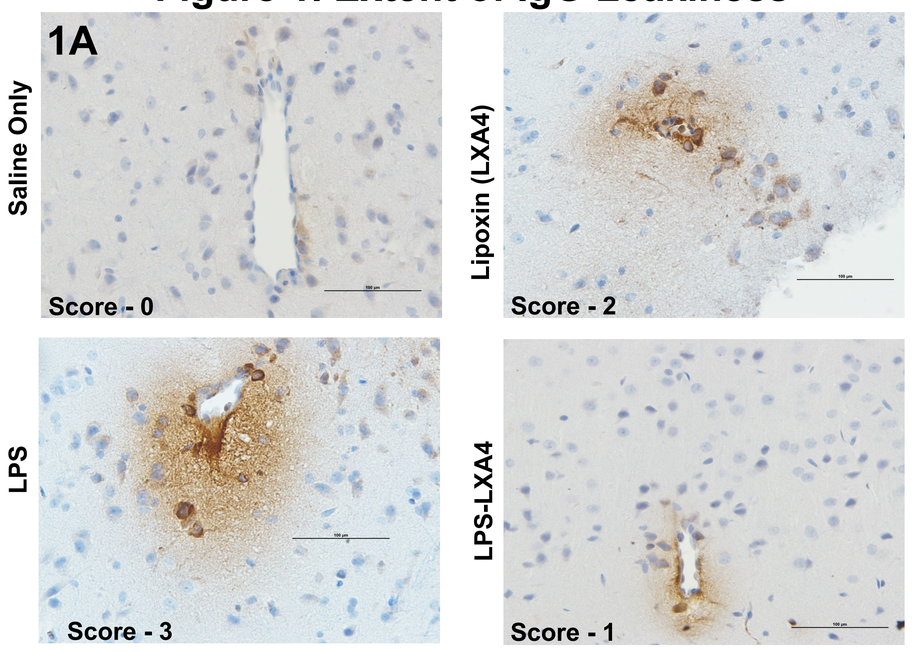
Methods: Nine-month-old female Sprague Dawley rats were given an intravenous (IV) injection of 15 mg/kg lipopolysaccharide (**LPS**) through the tail for inducing acute inflammation and BBB breach. After three hours, the rats were injected with 9 µg/kg LXA4 or Saline (Vehicle control). Four treatment groups were thus developed: LPS only, LPS/LXA4, LXA4 only, and Saline only. Animals were euthanized at 24 hours of LPS treatment and brain samples were processed for paraffin-embedded sections and immunohistochemistry. Sections comprising of hippocampus and cortical regions were selected for detection of impaired BBB as demonstrated by the extravasation of immunoglobulin G (**IgG**) and **IBA1 (microgliosis marker).** The area of the cerebral cortex and number of cortical blood vessels presenting with IgG extravasation were estimated and compared between treatment groups. Similarly, IBA1 immunoreactivity was quantified using **Color Deconvolution V9 tool of Aperio ImageScope** (Leica BIOSYSTEMS).

Results: LXA4 treatment following LPS injection demonstrated decrease in the extent of IgG leak compared to LPS only group. Likewise, we observed significant decrease in microgliosis in LPS-LXA4 group compared to LPS only.

Conclusion: These preliminary results demonstrate potential beneficial effects of LXA4 in reinstating BBB integrity and reducing neuroinflammation in rat model of acute BBB breach and inflammation.

Results

Figure 1. Extent of IgG Leakiness



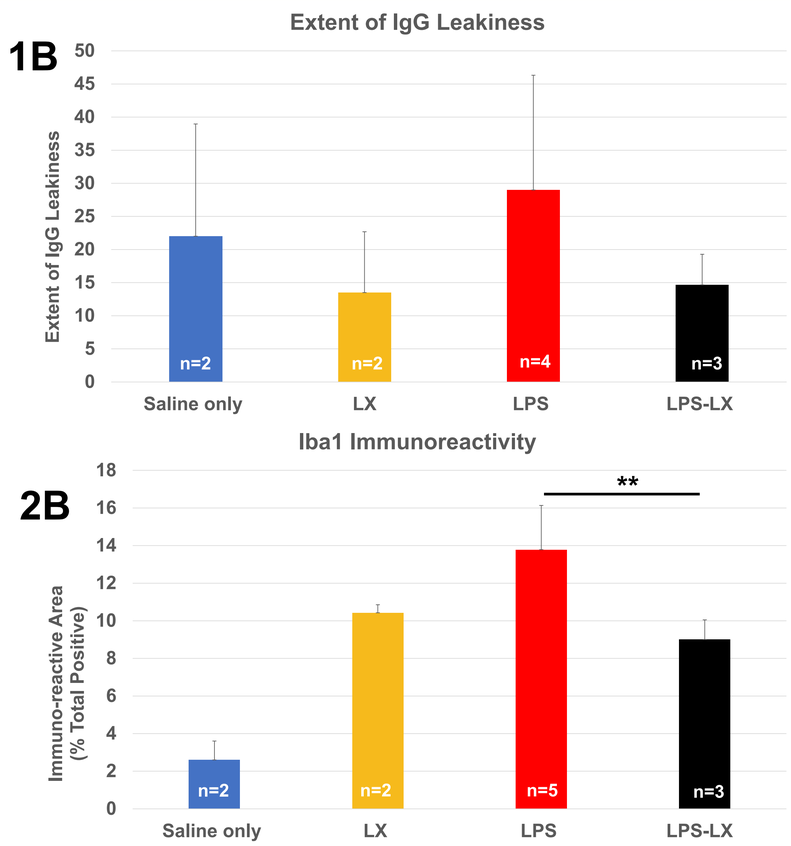


Figure 2. Iba1 Expression

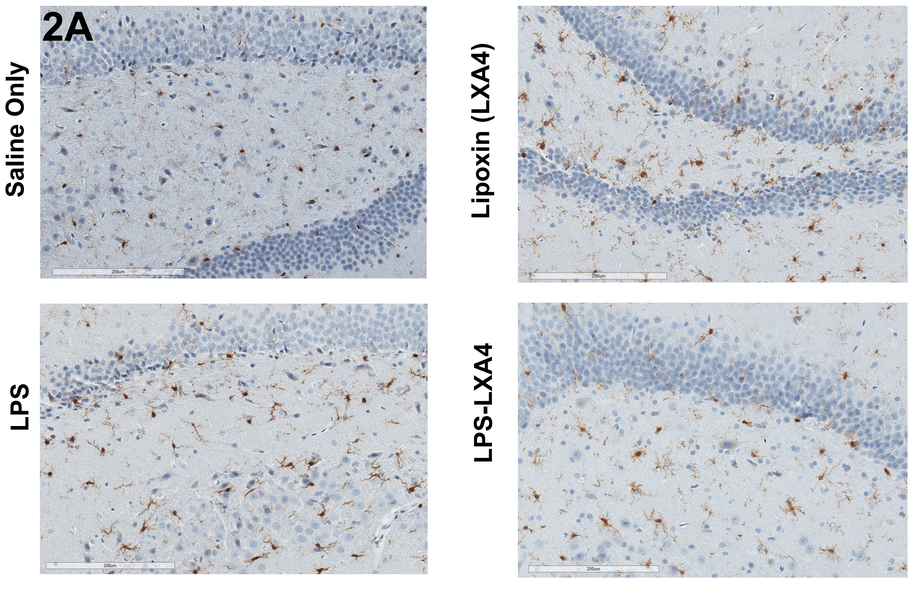


Figure 1. (A) Representative images from the hippocampal regions for the extent of IgG leakiness across the different treatment groups are shown. **(B)** IgG leakiness was quantified using a semi-quantitative scale of **0 to 3.** The score of IgG leakiness in also provided with the image at bottom left corner. Using this semi-quantitative approach, we observed reduced levels of IgG leakiness in the LPS-Lipoxin A4 group compared to LPS only. The difference however, failed to reach statistical significance, ***p = 0.29.*** Scale bars = 100 µm.

Figure 2. (A) Representative images from the hippocampal regions demonstrating Iba1 immunoreactivity across the different treatment groups. **(B)** Color Deconvolution V9 tool of Aperio ImageScope was used to estimate the percentage of total IBA1 immunoreactivity (brown color) in the entire brain section and the difference between LPS and LPS-LXA4 treatment groups attained statistical significance, ***p = 0.008*** **(**)**. Scale bars = 200 µm.

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