

EARLY CONSEQUENCES OF LYSOLECITHIN IN WHITE MATTER

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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease that targets oligodendrocytes and myelin and is associated with widespread neuronal and axonal injury thought to account for progression of disability. While several anti-inflammatory therapies are available for MS, they are ineffective in preventing neurological decline [1]. The lack of appropriate models hinders study of the causes of axonal injury. In MS lesions, damaged axons swell, forming spheroids, which present an early, reversible stage of axonal degeneration. This swelling can be distinguished by the accumulation of markers such as β -amyloid precursor protein (β -APP) [2].

Lysolecithin (LPC) is a detergent capable of inserting into lipid membranes, disrupting myelin sheaths, and is also a potent stimulator of microglia, which are highly activated in MS [3]. It was hypothesized that lysolecithin will induce axonal swelling and spheroid formation *in vivo*, similar to that found in MS, and that extended periods of LPC exposure will result in more severe and extensive axonal degeneration.

METHODS

YFP/Cox8 mice received stereotactic injections of 1% LPC or saline, along with Nuclear Blue to reveal the lesion site, into the ventral white matter tracts. Animals were sacrificed 4, 8, or 24 hours after injection, at which time they were perfused first with phosphate buffered saline and then with 4% paraformaldehyde. Spinal cord tissue was then removed, cryoprotected in 30% sucrose, frozen, and then sectioned in 20 μ m slices using a cryostat. After tissue had been sectioned, immunohistochemistry was used to label β -APP spheroids in damaged axons. Confocal microscopy was used to image lesions at 40X magnification and ImageJ was used to quantify the density of β -APP positive spheroids.

RESULTS

Treatment with LPC resulted in higher levels of damage to white matter, with the formation of more β -APP positive spheroids than when mice were injected with a control saline injection. The extent of LPC induced injury increased over time with significantly more β -APP positive spheroids at 24 hours as compared to 4 hours post injection.

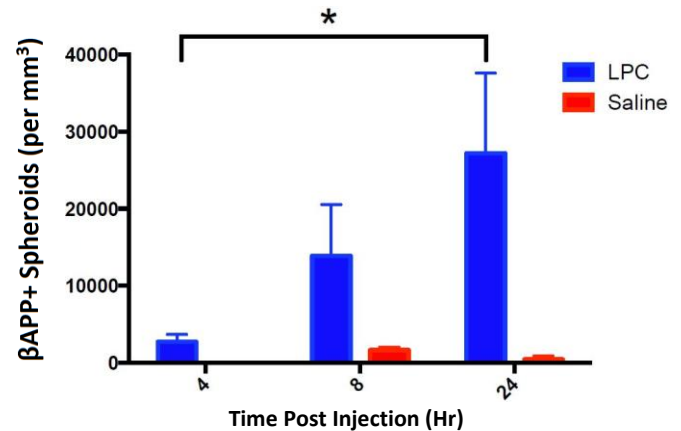


Figure 1. Density of β APP positive spheroids per mm³ in ventral white matter 4, 8, or 24 hours after stereotactic injection with 1% LPC (n=4-5) or saline (n=2-3). (Bars represent mean \pm SEM; 2-way ANOVA with Sidak's multiple comparisons was performed. *p < 0.05).

DISCUSSION AND CONCLUSIONS

This study reveals that LPC can cause axonal damage *in vivo* as demonstrated by the formation of prominent spheroids. Even in very early time-points, lysolecithin was capable of compromising axonal integrity and forming lesion pathology similar to that found in MS.

Given that LPC also induces demyelination and microglial activation, it is a useful model to study white matter injury. Examination of myelin injury, as well as neurofilament phosphorylation and levels of microglial activation in future research could help characterize other related mechanisms of injury in LPC-induced lesions.

Further understanding of the interrelationship between inflammation, axonal damage, and myelin injury after LPC-induced injury may inform potential mechanisms of MS progression and could aid in the development of new treatments to attenuate disability progression in MS.

REFERENCES

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