Preclinical evaluation of extra virgin olive oil and lutein-in-extra-virgin-olive-oil supplementation as a new avenue for prevention of retinopathy of prematurity

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Introduction

Retinopathy of prematurity (ROP) is a multifactorial retinal disorder that is a major cause of preventable childhood blindness among preterm infants who receive supplemental oxygen therapy. The relatively hypoxic environment after supplemental oxygen is removed triggers abnormal vascularization that often leads to intravitreal haemorrhage, retinal detachment and eventually vision loss. Since current treatment methods are often invasive and require long-term monitoring, the non-invasive nutraceutical strategy of using lutein, an anti-oxidising xanthophyll carotenoid, is investigated. Extra virgin olive oil (EVOO) and lutein-in-extra-virgin-olive-oil (LEVOO) is hypothesized to be safe and effective nutrient supplementation for mouse neonates to protect against ROP.

Objective

1. to investigate microglial activation in EVOO and LEVOO treated oxygen-induced-retinopathy pups
2. to ascertain the safety profile and beneficial role of EVOO and LEVOO in mouse neonates

Methods

Oxygen-induced retinopathy (OIR) is a well recognised animal model for mimicking ROP in humans. Postnatal day 7 mouse neonates are exposed to 75% oxygen and returned to room air 5 days later. EVOO and LEVOO are administered daily by intraperitoneal injection from postnatal day 12 onwards. Retinal vascularization peaks on postnatal day 17 which is when the mouse neonates are sacrificed and their eyes are collected. Retinal paraffin sections and flat mounts are then prepared for morphological examination.
Retinal microglia are detected by staining with an antibody against Iba-1. Cluster of differentiation 68 (CD68) is also used to identify activated microglia that indicate inflammation. The selection of fields of view in avascular area (AA), mid-peripheral area without neovascularization (non-tufts) and neovascularized areas (NV) are randomized. Confocal images of central and mid-peripheral zones from superficial and deep retinal vascular layers are then collected using a confocal laser scanning microscope at 200X magnification. The criteria for counting cell bodies include that their shapes must be round or hollow in the middle and of at least one cell size. Round shapes formed by cell tentacles are ignored.

Results

A total of 74 images were counted and analyzed with 2 or 3 images taken from the avascular area, 3 or 4 images taken from the non-tufts areas and 4 images taken from the NV areas from each retinal sample. Out of the 7 mice used, 3 were treated with EVOO while 4 were treated with LEVOO. The difference between microglial activation percentage of the EVOO and LEVOO groups is not statistically significant. For samples obtained from the AA area, the activation percentage of the LEVOO group (45%) is slightly lower than that of the EVOO group (50%). This difference is also seen in the non-tufts areas where the activation percentage of LEVOO group (52%) is lower than that of the EVOO group (54%). However, the activation percentage of the LEVOO group (75%) in the NV area is higher than that of the EVOO group (67%). The total number of microglial cells and activated microglia per mm2 of the LEVOO group is also slightly larger than that of the EVOO group. A probable reason for the lack of statistical significance despite the slight differences is that the sample size being investigated is too small.

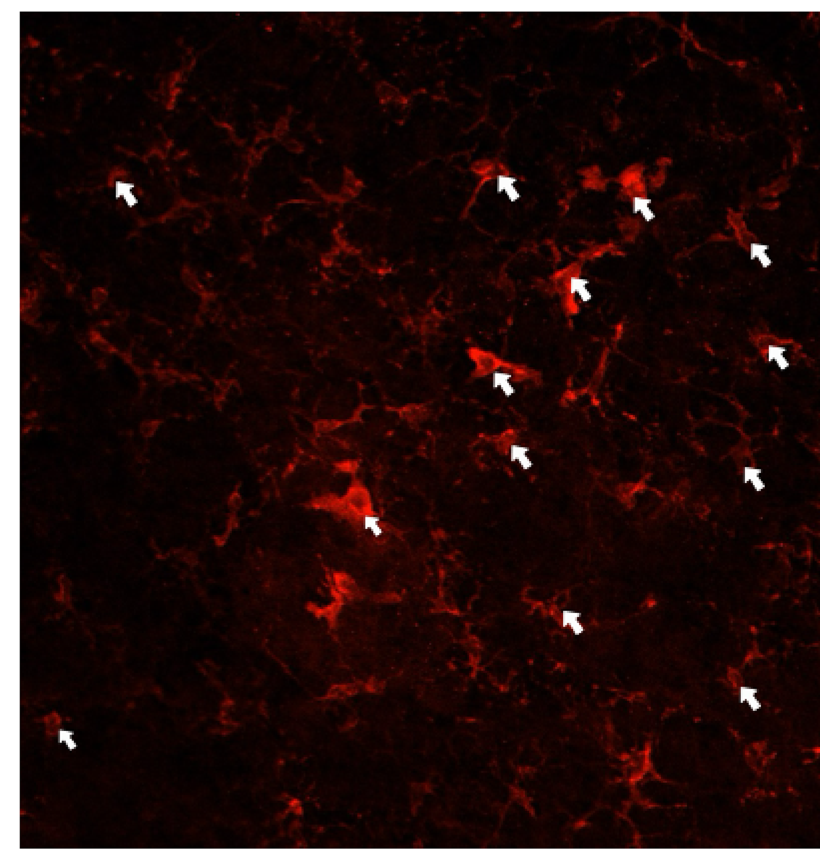

Figure 1: Example of microglial cell bodies stained by Iba-1, indicating al microglia.

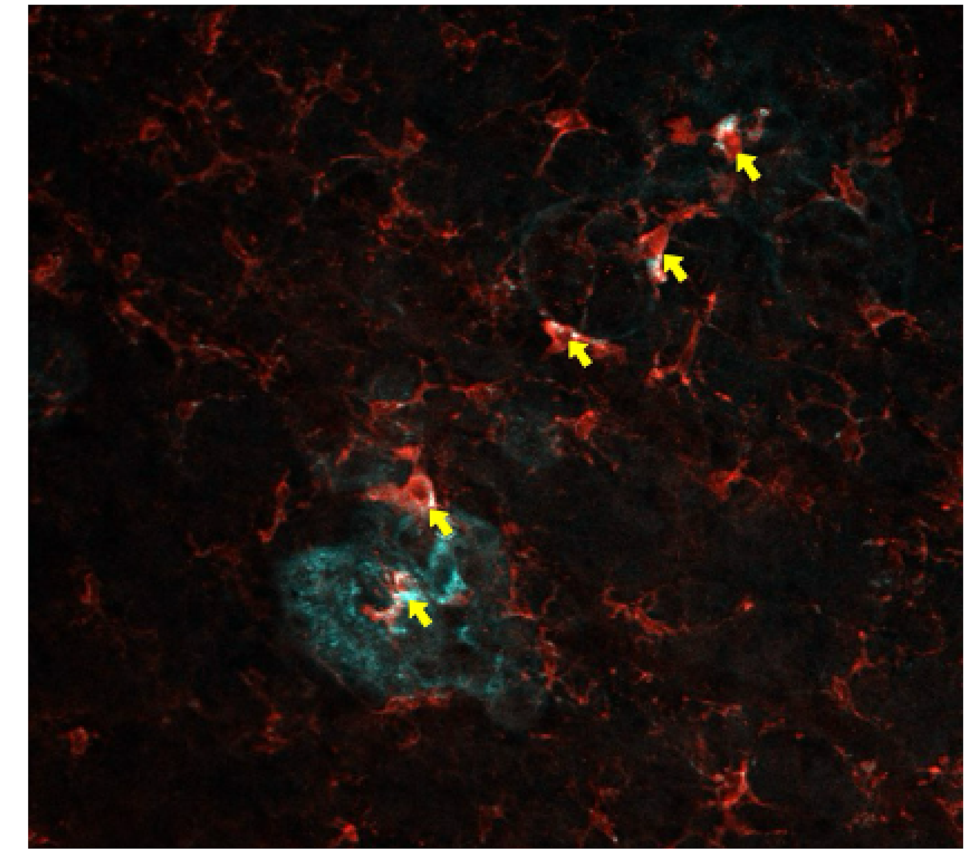

Figure 2: Example of microglical cell bodies co-stained with Ibal and CD68, indicating activated microglia.

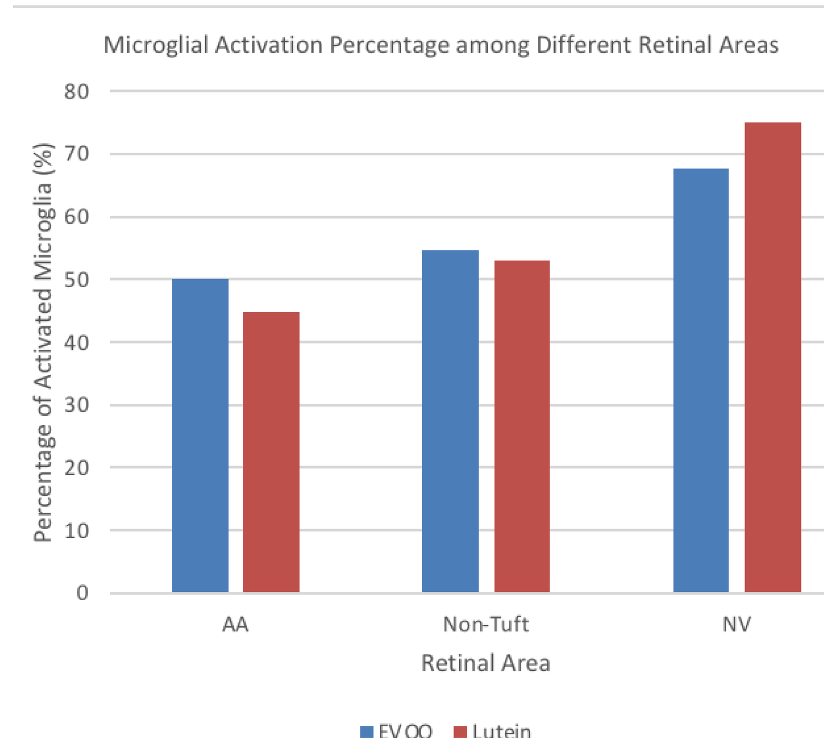

Figure 3: Bar chart showing differences between microglial activation in different areas.

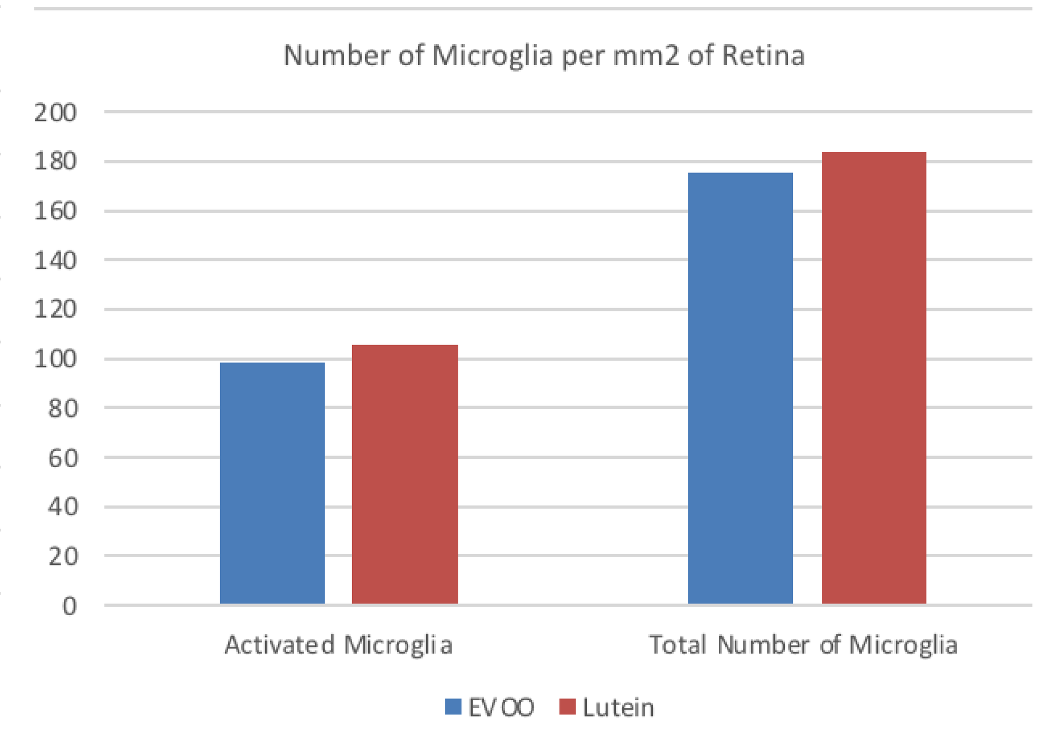

Figure 4: Bar Chart showing the number of microglia per mm2 of retina.

Skills Acquired

Retinal flat mount is a valuable micromanipulation skill acquired during this internship. The technique needed to dissect and manipulate a small structure under the microscope requires patience and practice and is a beneficial skill to obtain for a future medical career. Immunohistochemistry staining is also attempted for the visualization of different retinal cell layers. Understanding of the steps such as antigen retrieval, addition of primary and secondary antibodies as well as fluorescent examination and photo-taking are also essential takeaways from this internship. Preparation and staining samples with hematoxylin and eosin are also performed. Observing other laboratory work such as electroretinography, western blot and cell line transfer are also learning experiences that help develop a deeper understanding of how scientific research is conducted. Additionally, attending other events such as the Ophthalmology Grand Round, Distinguished Lecture and lab meetings are also valuable research-related academic exchange opportunities.

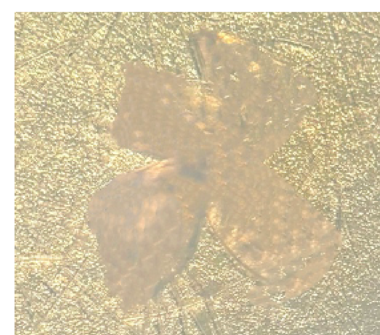

Figure 5: Retinal Flat Mount

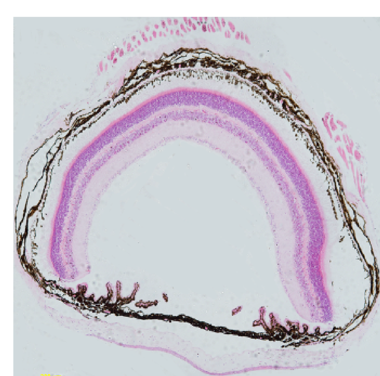

Figure 6: H&E stained sample

Conclusion

There was no significant difference between the EVOO and LEVOO groups. Further studies should include a larger sample size and conduct repeated counting. The study helps to assess the potential translational value of lutein-in-extra-virgin-olive-oil for better neonatal and pediatric care in Hong Kong by preventing or improving the outcome of retinopathy of prematurity.

Acknowledgement

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