Original Article

Omega-3 Supplementation Decreases Malondialdehyde, Vascular Endothelial Growth Factor Expression, Tumour Necrosis Factor Alpha, and Vascular Tuft in Oxygen-Induced Retinopathy

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ABSTRACT

Purpose: To determine whether omega-3 supplementation can reduce Malondialdehyde (MDA) levels, vascular endothelial growth factor (VEGF) expression, tumour necrosis factor-alpha (TNF-α) expression, and vascular tuft formation in oxygen-induced retinopathy (OIR) mouse models.

Study Design: Controlled laboratory experimental study.

Place and Duration of Study: Animal Laboratory Unit (ALU), Faculty of Medicine, Udayana University, from November 2019 to November 2020.

Methods: Thirty-six male Sprague Dawley rats, aged one week and weighing 10–12 g were included. Rats were exposed to 75 ± 5% oxygen from postnatal day 7 (P7) to P12 in a sealed oxygen chamber to induce vaso-obliteration. They were then returned to room air (20% oxygen) from P13 to P17 to allow hypoxia-driven pathological neovascularization. After that, rats were randomly assigned to either the treatment or control group. The treatment group received omega-3 fatty acids at a dose of 0.4 mg/g body weight, administered once daily via nasogastric tube from P13 to P17. Control animals did not receive supplementation and were maintained on standard laboratory diet. Retinas allocated for histological assessment were processed for immunohistochemical evaluation of VEGF, TNF-α, and vascular tuft formation.

Results: There was a statistically significant reduction in MDA levels, VEGF expression and TNF-α in the treatment group receiving omega-3 supplementation. The number of vascular tufts was notably lower following omega-3 administration, implying its potential to inhibit pathological angiogenesis.

Conclusion: Omega-3 unsaturated fatty acids exhibit antioxidant, anti-inflammatory, and anti-angiogenic properties that help suppress pathological neovascularization in oxygen-induced retinopathy (OIR).

Keywords: Retinopathy of Prematurity, Oxygen-Induced Retinopathy, Retinal Neovascularization, Ischemia, Retina, Fatty Acids, Omega-3, Dietary Supplements.

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INTRODUCTION

Retinopathy of Prematurity (ROP) represents a retinal vascular proliferative disease affecting premature infants. The leading risk determinants for ROP are extremely early gestational age and low birth weight. Administration of supplemental oxygen is often

provided as a crucial component in the care of premature infants, significantly contributing to the pathogenesis of ROP. It induces vaso-obliteration, halting blood vessel growth, and is followed by vasoproliferation, leading to the abnormal extension of new blood vessels toward the vitreous.[1]

Phase I of ROP, marked by vascular obliteration, begins when preterm infants adapt to the extrauterine environment. During this stage, oxygen supplementation inhibits retinal growth factors and induces regression of nascent vessels through vaso-obliteration.[1]

Animal models of oxygen-induced retinopathy (OIR) have been widely applied to study the mechanisms of ROP, especially during phases 1 and 2. The development of ROP phases 1 and 2 in these models has demonstrated the role of oxidative stress due to fluctuating oxygen exposure, which modulates VEGF expression.[2] Excessive oxidative stress activates several damaging pathways in retinal tissue, including inflammatory response. It also stimulates the production of inflammation-dependent reactive oxygen species (iROS).[3]

The phospholipid-rich retina is highly sensitive to injury from reactive oxygen species (ROS). Hyperoxia, hypoxia, and fluctuating oxygen conditions induce oxidative stress in retinal tissue by increasing ROS production, including superoxide anion (O_2^-) and hydroxyl radical (OH^-).[4] This ROS drive lipid peroxidation, resulting in malondialdehyde (MDA) formation and enhancing inflammation by suppressing peroxisome proliferator-activated receptor (PPAR) activity.

Omega-3 polyunsaturated fatty acids, particularly the active form docosahexaenoic acid (DHA), help prevent progression to phase II of ROP by curbing pathological neovascularization and inflammation. One mechanism is through modulating eicosanoid pathways: DHA and its precursor eicosapentaenoic acid (EPA) reduce the synthesis of prostaglandin E_2 (PGE_2) via the COX-2 enzyme, whereas PGE_2 would otherwise promote VEGF-driven angiogenesis.[5,6]

The human retina contains more DHA than any other tissue in the body. DHA, as a major omega-3 fatty acid, provides cytoprotective and neuroprotective benefits and plays a crucial role in regulating angiogenesis. Moreover, clinical studies indicate that giving fish oil (rich in omega-3) to very premature infants from the first day of life markedly lowers the incidence and severity of ROP, even though it does not entirely eliminate the need for laser treatment in babies who develop ROP.[7] The anti-angiogenic effect of omega-3 (particularly DHA) likely stems from its suppression of the VEGF signalling pathway, making omega-3 supplementation a promising strategy to prevent ROP progression.[1]

Based on the discussion above, further research is needed to examine whether omega-3 supplementation can reduce malondialdehyde (MDA) levels, vascular endothelial growth factor (VEGF) expression, tumour necrosis factor-alpha (TNF-α) expression, and vascular tuft formation in oxygen-induced retinopathy (OIR) mouse models.

METHODS

This experimental study employed a randomized post-test–only control group design using a Sprague Dawley rat model of oxygen-induced retinopathy (OIR). All procedures were conducted at the Animal Laboratory Unit (ALU), Faculty of Medicine, Udayana University. Analysis of retinal tissue, including ELISA and immunohistochemistry, was performed at the Integrated Biomedical Laboratory and Histology Laboratory of the same institution. Ethical approval was obtained from the Ethical Review Board of the Faculty of Medicine, Udayana University **(No. 107/UN14.2.2.VII.14/LP/2020).**

Male Sprague Dawley rats aged one week and weighing 10–12 g were eligible for inclusion. Rats exhibiting illness or failure to feed were excluded. A minimum of 16 animals per group was required; to compensate for potential attrition, an additional 10% was added, resulting in a total sample size of 36 rats.

Oxygen-Induced Retinopathy (OIR) model followed the Penn protocol. Rats were exposed to 75 ± 5% oxygen from postnatal day 7 (P7) to P12 in a sealed oxygen chamber to induce vaso-obliteration. They were then returned to room air (20% oxygen) from P13 to P17 to allow hypoxia-driven pathological neovascularization.

Upon return to normoxia (P13), rats were randomly assigned to either the treatment or control group. The treatment group received omega-3 fatty acids at a dose of 0.4 mg/g body weight, administered once daily via nasogastric tube from P13 to P17. Control animals did not receive supplementation and were maintained on standard laboratory diet.

On P18, all animals were anesthetized and

Table 1: *Descriptive data on MDA levels, VEGF expression, TNF- and vascular tuft in the treatment and control groups.*

Variable	Group	N	Average	Standard Intersection	Average Difference
MDA (nmol/mL)	Treatment	18	1.56	0.27	0.41
	Control	18	1.97	0.49	
VEGF	Treatment	18	1.87	0.46	0.34
	Control	18	2.21	0.25	
TNF-α	Treatment	18	10.78	3.42	9.72
	Control	18	20.5	3.9	
Vascular tuft	Treatment	18	8.78	2.46	6.33
	Control	18	15.11	3.88	

euthanized, followed by bilateral enucleation. Retinas designated for biochemical analysis were stored in phosphate-buffered saline (PBS) for quantification of malondialdehyde (MDA) levels using ELISA. Retinas allocated for histological assessment were fixed in Bouin's solution and processed for immunohistochemical evaluation of VEGF, TNF-α, and vascular tuft formation.

Data were analysed using SPSS version 24. Descriptive statistics were generated for all outcome variables. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance using Levene's test. No missing data were reported. Intergroup comparisons were performed using the independent t-test for normally distributed data or the Mann–Whitney U test for non-normal data. Statistical significance was set at $p < 0.05$ with a 95% confidence interval.

RESULTS

A total of 36 mice were randomly divided into two groups, with 18 in the control group and 18 in the treatment group. The descriptive analysis aimed to provide a clear overview of the distribution of research variables across both groups.

As presented in Table 1, differences were observed in the mean levels of MDA, VEGF expression, TNF-α expression, and vascular tuft formation between the treatment and control groups. Notably, the control group exhibited higher levels of these markers compared to the treatment group.

Statistical analysis revealed a significant reduction in MDA levels in the treatment group receiving omega-3 supplementation, suggesting that omega-3 unsaturated fatty acids help lower retinal MDA levels in OIR model rats (Table 2). Similarly, VEGF expression was significantly lower in the treatment group, indicating that omega-3 may suppress VEGF expression in the OIR model.

Table 2: *Comparability test of MDA levels using the Mann-Whitney U test.*

	Group	N	Mean Rank	Z	p
MDA (nmol/mL)	Treatment	18	14.44	-2.31	0.021
	Control	18	22.56		

Further analysis showed a significant decrease in TNF-α expression in the treatment group, suggesting a role for omega-3 unsaturated fatty acids help mitigate inflammation by decreasing the TNF-α expression. Additionally, the number of vascular tufts was notably lower following omega-3 administration, implying its potential to inhibit pathological angiogenesis (Table 3).

Table 3: *Comparability test of VEGF, TNF-α, and vascular tuft using independent t-test.*

	Group	N	Average	SB	95% CI	p
VEGF	Treatment	18	1.87	0.46	0.09 - 0.59	0.009
	Control	18	2.21	0.25		
TNF-α	Treatment	18	10.78	3.42	7.42 - 12.21	<0.05
	Control	18	20.5	3.89		
Vascular tuft	Treatment	18	8.78	2.46	4.12 - 8.55	<0.05
	Control	18	15.11	3.88		

DISCUSSION

Our study found that retinal MDA levels were lower in the omega-3 supplemented group than in controls, likely because omega-3 fatty acids boost the eye's own antioxidant defences. Antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase neutralize ROS in the retina and thereby prevent oxidative stress. Omega-3 appears to enhance the expression of these antioxidant enzymes by activating genes regulated by the transcription factor Nrf2.[8]

Recent clinical data from Pakistan also report a substantial burden of retinopathy of prematurity among premature infants, emphasizing the critical role of oxygen exposure and oxidative stress in early retinal vascular injury, which supports the importance of antioxidant mechanisms observed in our OIR model.[9]

Furthermore, VEGF levels in the treatment group receiving omega-3 supplementation were significantly lower than in the control group. This indicates that omega-3 unsaturated fatty acids influence the angiogenesis pathway by inhibiting VEGF. VEGF, primarily produced by Müller cells in the retina, has neuroprotective functions under normal conditions but can promote pathological angiogenesis when overexpressed. The suppression of VEGF expression by omega-3 is believed to occur through HIF-1 ubiquitination, leading to its degradation. This prevents HIF-1 translocation to the nucleus, thereby inhibiting its activation and subsequent regulation of hypoxia response element (HRE) genes involved in angiogenic factor expression. Inhibition of HIF-1 can occur at multiple levels, including nuclear translocation, transcription, translation, and DNA binding.[10]

A study by Zhuang *et al,* found that EPA and DHA did not induce vascular endothelial cell death but instead suppressed VEGF mRNA expression under hypoxic conditions.[11] This inhibition of VEGF further reduces endothelial cell proliferation and slows the wound-healing process. The anti-angiogenic properties of EPA and DHA, demonstrated through VEGF suppression, suggest potential benefits for diseases associated with angiogenic factors, such as chronic inflammation and retinopathy. Elevated VEGF in retinopathy compromises the blood-retinal barrier, leading to abnormal vascular formation. However, the potential side effects of EPA and DHA in revascularization therapy require further investigation.[11]

We also found that retinal TNF-α expression was significantly lower in the omega-3 group than in controls. This suggests that omega-3 fatty acids inhibit TNF-α, a key inflammatory mediator, likely by modulating intracellular signalling pathways. Specifically, omega-3 can suppress activation of NF-κB, a central transcription factor that upregulates inflammatory cytokines and drives the expression of pro-inflammatory genes like TNF-α and IL-6.[12]

Our findings are consistent with those of Connor *et al*, who studied dietary omega-3 in an OIR mouse model.[2] In that study, mice were fed a diet containing 2% total fatty acids as either omega-3 or omega-6 and then exposed to hyperoxic conditions following the OIR protocol. Connor and colleagues observed that lowering the omega-6/omega-3 ratio reduced pathological angiogenesis: mice on the omega-3, rich diet showed less abnormal retinal neovascularization. Greater omega-3 intake was associated with smaller avascular retinal areas, more robust regrowth of normal blood vessels, and a weaker hypoxic stimulus for neovascularization. The authors suggested that omega-3, derived mediators (neuroprotectin D1, resolvin D1, resolvin E1) help inhibit aberrant vessel growth, possibly by suppressing inflammatory cytokines such as TNF-α.[2]

Vascular tuft formation, indicative of ischemic retinopathy, represents pathological angiogenesis and is a hallmark of retinopathy treatment. In this study, the number of vascular tufts was significantly lower in the omega-3 treatment group compared to the control group. This suggests that omega-3 has an inhibitory effect on pathological neovascularization by modulating angiogenic pathways. The suppression of vascular tuft formation is thought to occur through inhibition of VEGF, PDGF, and prostaglandin E2, as well as through the downregulation of inflammatory mediators like TNF-α.[12,13]

NF-κB is a pivotal transcription factor in inflammatory responses. Activated NF-κB has been observed in the retinal vasculature of diabetic patients as well as in diabetic animal models. In human retinal endothelial cells, TNF-α upregulates the adhesion molecules ICAM-1 and VCAM-1, which contribute to angiogenesis.[12] TNF-α also drives NF-κB to move into the nucleus and bind to the VCAM-1 gene promoter, amplifying the inflammatory response. DHA, however, can counter these effects by preventing NF-κB's nuclear translocation and DNA binding, thereby reducing inflammation.[10,12,14]

A limitation of this study is the absence of pre-treatment baseline measurements before omega-3 supplementation, which may have provided a more detailed view of biomarker changes over time. Additionally, this study was performed using a single-centre animal laboratory model, which may limit generalizability. Furthermore, we did not evaluate dose–response relationships or long-term outcomes

beyond the OIR induction period. Future research should incorporate multi-centre designs, baseline measurements, and extended follow-up intervals to enhance external validity and mechanistic understanding.

CONCLUSION

Omega-3 unsaturated fatty acids exhibit antioxidant, anti-inflammatory, and anti-angiogenic properties that help suppress pathological neovascularization in oxygen-induced retinopathy (OIR). Their effects reduce oxidative stress, inhibit VEGF-mediated angiogenesis, and modulate inflammatory pathways, ultimately limiting vascular tuft formation. These findings highlight the potential therapeutic role of omega-3 in preventing retinal neovascularization associated with OIR.

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Patient's Consent: Researchers followed the guidelines set forth in the Declaration of Helsinki.

Conflict of Interest: Authors declared no conflict of interest.

Ethical Approval: The study was approved by the Institutional review board/Ethical review board **(107/UN14.2.2VII.14/LP/2020)**.

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Authors Designation and Contribution

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