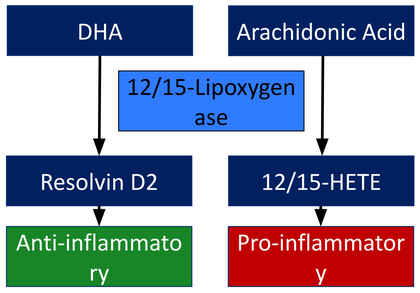
Effects of Resolvin-D2 in Lipopolysaccharide-induced Retinal Inflammation

Min Young Kim1,2,3, Mohamed Moustafa2,3, Sonali Sharma2,3, Ahmed Elmarakby4,5, Mohamed Al-Shabrawey1,2,3,*

1 Oakland University William Beaumont School of Medicine, Rochester, MI, USA, 2 Eye Research Center, Oakland University William Beaumont School of Medicine (OUWB-SOM), Rochester, MI, USA, 3 Eye Research Institute, Oakland University, Rochester, MI, USA, 4 Department of Oral Biology and Diagnostic Sciences, Dental College of Georgia, Augusta University, Augusta, GA, USA, 5 Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta University, Augusta, GA, USA

Introduction

12/15-lipoxygenase (12/15-LO) is a double-edged sword which has the ability to generate pro-inflammatory and anti-inflammatory mediators depending on the type of available substrate. For example, docosahexaenoic acid (DHA) , a biologically active ω−3 poly unsaturated fatty acid (PUFA), is a potential substrate for 12/15-LO to produce anti-inflammatory and antioxidant properties by producing specialized pro-resolving lipid mediators. On the other hand, arachidonic acid (AA, ω−3 PUFA) is a substrate for 12/15-LO that produces pro-inflammatory 12- and 15-HETEs. We recently demonstrated a significant increase in the circulating levels of 12/15-LO pro-inflammatory metabolites 12/15-HETEs and a contrary decrease in the levels of the anti-inflammatory metabolite resolvin-D2 (RvD2) in lipopolysaccharide (LPS)-treated mice. Dietary supplement of DHA restored normal levels of RvD2 and attenuated LPS-induced acute kidney injury in mice.



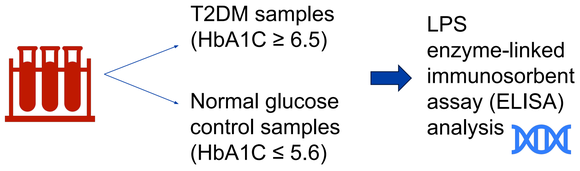
Aims and Objectives

- Goal: To examine whether RvD2 and/or DHA mitigates retinal inflammation using the LPS-induced experimental inflammation model. As retinal vascular inflammation can lead to pathologic changes to cause blindness, finding possible therapeutic strategy that is non-invasive will be beneficial in clinical settings.
- We hypothesize that LPS promotes pro-inflammatory bioactive lipid production in the retina through increased 12/15-LO activity, and that RvD2 and/or DHA counteracts this effect.

Methods

Human Specimen

Human blood samples were collected from subjects recruited from the University of Sao Paulo School of Dentistry in Brazil between January 2014 and May 2015.



Methods (continued)

Animal Design

Wild Type (WT)	12/15-LO Knockout (KO)
1. Saline (Control) 2. LPS (intraperitoneal (ip) 4 mg/kg) 3. LPS + RvD2 (100 ng/mouse ip) 4. LPS + DHA (50 mg/kg, ip) 5. LPS + Baicalein (20 mg/kg, ip)	1. Saline (Control, ip) 2. LPS (4 mg/kg, ip)

12/15-LO Knockout mice were used to determine DHA's a direct effect on inflammation in the absence of RvD2.

- **D 1-3**: • Treatment • DHA, RvD2, Baicalein
- **Day 4**: • LPS injection • Treatment • DHA, RvD2, Baicalein
- **D 5-6**: • Treatment • DHA, RvD2, Baicalein
- **Day 7**: • Termination • Tissue and Plasma collection

Each group of mice were age and weight matched, 12-15 week male mice from The Jackson Laboratory.

From collected tissue and plasma samples of each group, RNA expression levels of 12/15-LO, TNF-a, IL-6, IL-10, IL-1b, and ICAM-1 were analyzed with RT-PCR.

Human Retinal Endothelial Cells (HREC) cell culture

HREC Treatment Groups
1) Condition media (control)
2) TNFα only (20ng/ml)
3) RvD2 only (50nM)
4) TNFα (20ng/ml) + RvD2 (50nM)

Days 1-2: Treated with RvD2
Day 3: TNFα insult
- @6 hours, culture media collected
- @24 hours, culture media and cell lysates collected

Performed cytokinesis array and western blot analysis

in vitro HREC

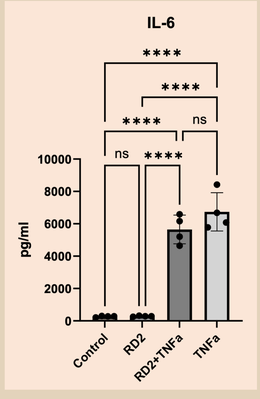


Figure 4 (left). Both RvD2+TNFα and TNFα groups had significantly increased IL-6 released to the cell media twenty-four hours after the TNFα insult. Treatment with RvD2 before TNFα did not have significant therapeutic effects on the level of IL-6 released from HREC compared to TNFα group. **** P<0.0001. n=4

Results and Discussion

Human Specimen

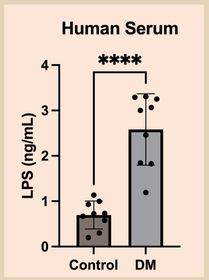


Figure 1. Significantly (P<0.0001) higher amount of LPS was detected in serum samples of patients with T2DM compared to patients with no T2DM with LPS ELISA.

Animal Design

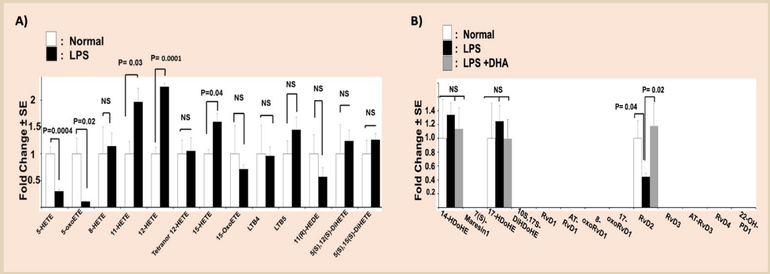


Figure 2. LPS induced isolated bioactive lipid level fold changes in the *in vivo* mice model. **(A)** LPS significantly increased the plasma level of 12/15-LO metabolites of arachidonic acid (12/15-HETEs). **(B)** LPS reduced the plasma levels of RvD2, a derivative of DHA by 12/15-LO. Administration of DHA to LPS-treated mice restored the normal levels of RvD2. n=4-5

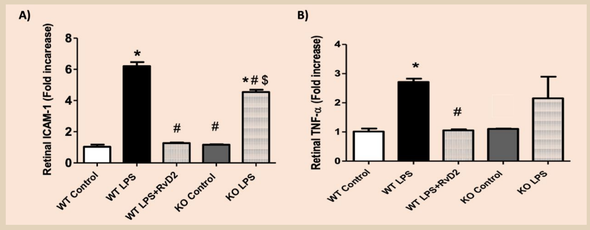


Figure 3 (left). RvD2 is the key player in providing anti-inflammatory response in LPS-induced retinal inflammation. In wild-type (WT) mice groups, LPS induced significant upregulation of ICAM-1 **(A)** and TNFα **(B)**, which was significantly reduced by RvD2 treatment. Deletion of 12/15-LO did not show significant effects on their retinal levels. *P<0.05 vs control, # P<0.05 vs LPS, $ P<0.05 vs control. n=5-6.

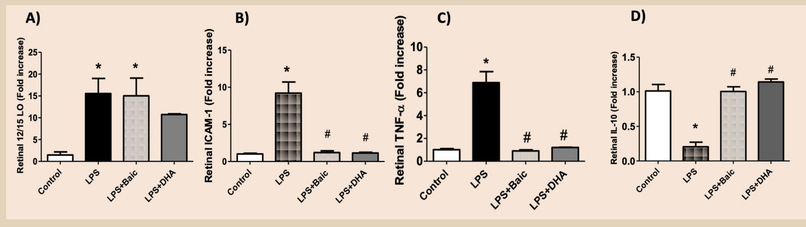


Figure 4. RT-PCR analysis of retinal 12/15-LO and inflammatory markers mRNA. Treatment of mice with LPS induced significant increases in retinal 12/15-LO level. Even with 12/15-LO inhibitor baicalein, the result shows no change in 12/15-LO since baicalein inhibits existing 12/15-LO enzyme rather than inhibiting its synthesis **(A)**. Administration of baicalein and DHA significantly abrogated LPS-induced upregulation of retinal ICAM1 **(B)** and TNFα **(C).** Interestingly, LPS significantly reduced the anti-inflammatory cytokine IL10 which was restored to normal levels by baicalein and DHA **(D).** *P<0.05 vs control, # P<0.05 vs LPS. n=5-6.

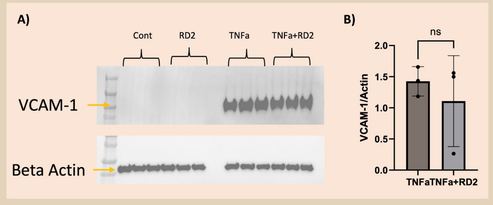


Figure 5. RvD2 treatment did not reduce TNFα-induced VCAM-1 protein expression in HREC *in vitro* model inflammatory response. **(A)** Beta actin control protein showed adequate protein concentration collected from HREC cell lysates. VCAM-1 was detected in TNFα and TNFα+RvD2 groups. **(B)** No significant difference in HREC regulation of VCAM-1 expression between TNFα and TNFα+RvD2 groups. n=3.

Conclusion

Increased LPS in diabetic patients is consistent with its role in DR. RvD2 and DHA are potential therapies for retinal inflammation. Lack of protection in HREC suggests that retinal cells other than HREC may be responsible for initiating retinal inflammatory responses in diseases such as in DR.

References

SCAN ME

Acknowledgements

This research was funded by the National Eye Institute grants R01EY030054-01A1, Oakland University and Oakland University William Beaumont School of Medicine (M.A.). We are glad to use this opportunity to acknowledge OUWB's EMBARK research program for encouraging student research.