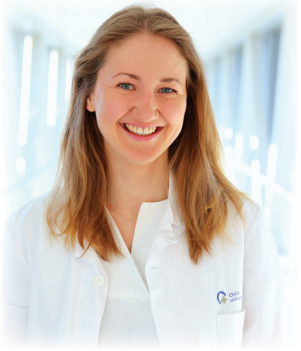
Can a Fish a Day Keep the Doctor Away?

Omega-3 fatty acid–derived resolvins on the ocular surface



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On May 4, 2023, Nora Botten defended her thesis "Specialized pro-resolving lipid mediators resolvin D1, resolvin D2, and resolvin E1 in the maintenance of ocular-surface homeostasis and in prevention of ocular-surface inflammatory disease" at the University of Oslo (UiO), Institute of Clinical Medicine. The research was carried out at the Department of Medical Biochemistry, Oslo University Hospital (OUH), and Schepens Eye Research Institute, Harvard Medical School. Her main supervisor was Tor Paaske Utheim, Department of Ophthalmology, OUH, with co-supervisors Darlene Ann Dartt, Schepens Eye Research Institute, Harvard Medical School, and Kim Alexander Tønseth, Department of Plastic and Reconstructive Surgery, UiO.

Key points:

- Goblet cells in the conjunctiva secrete mucins into the tear film to maintain ocular-surface health.
- Omega-3-derived resolvins contribute to the resolution of inflammation and the return to homeostasis.
- Resolvins activate multiple intracellular signaling pathways in goblet cells that, in turn, stimulate the secretion of mucins into the tear film.
- Resolvins regulate goblet cell function and could represent a novel approach for treating ocular-surface inflammation.

Introduction

One characteristic of ocular-surface inflammatory diseases, such as dry eye disease and allergic conjunctivitis, is the dysregulation of goblet cells in the conjunctiva, leading to the over- or under-secretion of glycoproteins into the tear film. Goblet cell secretion is tightly regulated, and either increased or decreased secretion can lead to inflammation. Specialized pro-resolution mediators, such as omega-3 fatty acid–derived resolvins, contribute to the resolution of inflammation. In preclinical studies, resolvins have shown promising actions in the treatment of inflammatory diseases, including ocular-surface inflammatory disease. Thus, this study aimed to determine whether and how resolvins regulate secretion from healthy conjunctival goblet cells.

Methods

Goblet cells from rat and human conjunctiva were cultured and used for experiments. Receptor expression was determined using PCR, siRNA, Western blotting, and immunofluorescence microscopy. Intracellular signaling pathways activated by resolvins were then investigated using two different methods. First, we measured the intracellular Ca^{2+} concentrations in goblet cells using Fura-2. Second, we measured the secretion of high-molecular-weight glycoproteins from goblet cells using an enzyme-linked lectin assay with the lectin horseradish peroxidase–conjugated UEA-1. In each experiment, half of the cell culture dishes were incubated with pharmacological inhibitors known to inhibit one specific intracellular signaling molecule. Goblet cells in the cell culture dishes were then stimulated with one resolvin.

Results

Resolvins in the D and E series activated different receptors on the goblet cell surface to trigger multiple intracellular signaling pathways. The activation of intracellular signaling increased Ca^{2+} or stimulated the secretion of mucins into the tear film. The resolvins that were investigated in this project activated many of the same intracellular signaling molecules, such as phospholipase C (PLC). PLC produces molecules such as inositol trisphosphate, which releases Ca^{2+} from intracellular stores in the endoplasmic reticulum within goblet cells. We also discovered differences in the intracellular signaling pathways activated by resolvins. Two examples are the cyclic AMP signaling pathway and activation of the epidermal growth factor receptor, which are activated by only some resolvins, but not others, to increase Ca^{2+} or stimulate secretion into the tear film.

Conclusion

Resolvins activate multiple intracellular signaling pathways to stimulate secretion in healthy goblet cells from the conjunctiva. Dysregulation of mucin secretion from goblet cells is a characteristic of ocular-surface inflammatory diseases. Regulation of goblet cell secretion is important to maintain ocular-surface homeostasis and prevent inflammation. Resolvins could represent a novel approach to treat inflammation on the ocular surface.

Remaining questions:

- If investigated in vivo, do resolvins regulate goblet cell function and stimulate the secretion of mucins into the tear film?
- Would treatment with resolvins be more effective at reducing inflammation on the ocular surface than today's treatments for dry eye disease and allergic conjunctivitis?

References

1. Botten N, et al. Resolvin D2 elevates cAMP to increase intracellular [Ca^{2+}] and stimulate secretion from conjunctival goblet cells. *FASEB J*. 2019 Jul;33(7):8468-8478.
2. Botten N, et al. Resolvin D2 uses multiple Ca^{2+} -dependent signaling pathways to stimulate mucin secretion in rat and human conjunctival goblet cells. *J Cell Physiol*. 2022 Oct;237(10):3816-3833.
3. Kaye R, Botten N, et al. Resolvin D1, but not resolvin E1, transactivates the epidermal growth factor receptor to increase intracellular calcium and glycoconjugate secretion in rat and human conjunctival goblet cells. *Exp Eye Res*. 2019 Mar;180:53-62.