since 2-deoxy-D-glucose can be incorporated into viral glycoproteins, results obtained with this compound may not be comparable to those obtained using inhibitors such as tunicamycin.

In this report, neither tunicamycin nor 2-deoxy-D-glucose proved effective in the treatment of stromal keratitis in rabbit corneas. One possible explanation is that glycoprotein production is not the sole determinant of stromal disease. Some evidence suggests a multiple antigenic etiology. Results of an in vitro study showed that in the presence of tunicamycin, antigenically-active, lower molecular-weight polypeptides were found that were antigenically and structurally related to the glycosylated proteins. In another study, a class of glucosamine-containing heterosaccharides (MW less than 3000) not present in DG-free HSV-infected cells was accumulated in the presence of 2-deoxy-D-glucose. These lower molecular-weight proteins and/or heterosaccharides also may contribute to the antigenic stimulus for the production of stromal disease, thereby negating the effect of glycoprotein inhibition. Finally, it is not known certainly that tunicamycin and 2-deoxy-D-glucose can inhibit glycoprotein production when applied topically in the kind of dosage schedule used here. Further work, possibly involving recombinant HSV strains with well-characterized glycoproteins production, may be needed to confirm the findings suggested by the results obtained here.

Key words: herpes simplex virus, stromal keratitis, tunicamycin, 2-deoxy-D-glucose, rabbit model

From the Lions Eye Research Laboratories, LSU Eye Center, Louisiana State University Medical Center School of Medicine, New Orleans, Louisiana. *Dr. Raju’s permanent address is: Department of Ophthalmology, West Virginia University Medical Center, Morgantown, WV. Supported in part by USPHS grants EY02672 and EY02377 from the National Eye Institute, Bethesda, Maryland. Submitted for publication: May 5, 1983. Reprint requests: H. E. Kaufman, MD, LSU Eye Center, 136 South Roman Street, New Orleans, LA 70112.

References

Human Anterior Uvea Synthesizes Lipoxygenase Products from Arachidonic Acid
Prasad S. Kulkarni, Ana V. Rodriguez, and B. D. Srinivasan

Arachidonic acid is metabolized into biologically active prostanoids, thromboxanes, and lipoxygenase products, leukotrienes. In the present study, the ability of human anterior uvea to synthesize lipoxygenase products from [3H]-radio labelled arachidonic acid is assessed. Following cyclooxygenase inhibition by indomethacin, human anterior uvea, similar to rabbit conjunctiva and anterior uvea, synthesizes chemotactic products 12-hydroxeyicosatetraenoic acid (HETE) and 5,12-DIHETE, indicating the presence of both 5- and 12-lipoxygenase enzyme activities. Invest Ophthalmol Vis Sci 25:221–223, 1984

Arachidonic acid is not only converted into primary stable prostaglandins (PGs) such as PGE2, PGF2α, and PGD2 via the cyclooxygenase pathway, but also into
unstable but potent bioactive compounds like PGI₂ (prostacyclin) and thromboxane-A₂ (TXA₂) in various tissues, including ocular tissues such as rabbit conjunctiva and anterior uvea. Arachidonic acid is also converted into another biologically active class of compounds, the so-called leukotrienes and hydroxyeicosatetraenoic acids (HETEs) via the 5- and 12-lipoxygenase pathways in various tissues. The 12-lipoxygenase enzyme pathway converts arachidonic acid into 12-HETE, which is known to be chemotactic for PMNs, whereas the 5-lipoxygenase enzyme pathway converts arachidonic acid into leukotriene B₄ (5S-12R dihydroxyeicosatetraenoic acid: 5,12-DiHETE), a potent chemotactic agent for polymorphonuclear leukocytes, and leukotrienes C₄, D₄, and E₄, whose mixture is now known to be the slow-reacting substance (SRS-A) of anaphylaxis, a mediator of asthma and certain allergic reactions.

Lipoxygenase products, such as 12-HETE, 5-HETE, and 5,12-DiHETE, are synthesized by rat, rabbit, dog, guinea pig, cat and monkey conjunctiva and anterior uvea. In the present study, we determined whether or not human anterior uvea has the ability to synthesize lipoxygenase products from arachidonic acid.

**Materials and Methods.** Human anterior uveal tissues (nondiabetic) were obtained from the National Diabetic Research Interchange (Philadelphia) and stored in the deep freezer at −100°C temperature until used. Tissues were defrosted and chopped and, in order to inhibit the cyclooxygenase pathway, pretreated with indomethacin (10 μg/ml) for 30 min. Previously, in order to study the synthesis of lipoxygenase products from ¹⁴C-arachidonic acid in rabbit anterior uvea, a 30-min incubation period was used. These studies showed that this tissue did not synthesize lipoxygenase products. In a later study, however, when rabbit anterior was incubated for a longer time (1-2 hrs), it synthesized detectable amounts of all lipoxygenase products from ¹⁴C-arachidonic acid. Therefore, human anterior uveal tissues were incubated with ¹⁴C-arachidonic acid (200 nCi, specific activity 40 mM/mCi, New England Nuclear, MA) in Krebs-Henseleit solution at 37°C for 2 hrs. In order to confirm and compare the synthesis of lipoxygenase products, we simultaneously incubated equal numbers of indomethacin-treated rabbit conjunctival tissues (also stored at −100°C prior to use) with the radiolabelled substrate for 2 hrs. At the end of the incubation period, the reaction was stopped by ice-cold acetone containing 1 N HCl. The procedure of extraction and separation of lipoxygenase products by thin-layer chromatography (TLC) has been described previously. The developed chromatogram was exposed to x-ray film (Kodak XR-7) for 15 days. The film was then developed and with the TLC plate and autoradiogram accurately superimposed, areas of lipoxygenase products were marked and scraped off the plate for quantitative measurements of ¹⁴C-content by liquid scintillation counting.

**Results.** Figure 1 is a representative autoradiogram of one out of eight experiments showing the radiolabelled spots of arachidonic acid (Rf = 1), 12-HETE (Rf = 0.78), 5-HETE (Rf = 0.5), 5,12-DiHETE (Rf = 0.12), and origin-containing prostaglandins and phospholipids synthesized by indomethacin-treated human anterior uvea and rabbit conjunctiva. Table 1 illustrates the synthesis of various lipoxygenase products from ¹⁴C-arachidonic acid by indomethacin-treated human anterior uvea.

**Discussion.** The results indicate that indomethacin-treated human anterior uvea synthesizes 12-HETE and...
5,12-DiHETE, suggesting the presence of both 5 and 12 lipoxygenase enzyme activities in this tissue. However, human anterior uvea has considerably less capacity (4-5-fold) than rabbit anterior uvea to synthesize lipoxygenase products from arachidonic acid. Since the present data indicate the presence of 5-lipoxygenase activity and SRS is a 5-lipoxygenase product, we are in the process of determining the formation of SRS-like activity in human anterior uvea using the bioassay technique. Our preliminary data with indomethacin-treated human anterior uvea suggest that these tissues also synthesize SRS-like activity similar to rabbit ocular tissues.

In different experimental animal models such as carageenin-induced inflammation, corneal epithelial denudation, and bovine serum-induced uveitis, low doses of nonsteroidal antiinflammatory drugs such as indomethacin that inhibit only cyclooxygenase pathway potentiate PMN chemotaxis. Higgs et al. suggested that low doses of indomethacin inhibit only cyclooxygenase but facilitate the synthesis of lipoxygenase products that are chemotactic for PMNs. If lipoxygenase product synthesis by human anterior uvea plays a role in inducing inflammatory cell infiltration in certain clinical inflammatory conditions, then the use of nonsteroidal antiinflammatory drugs may exacerbate the inflammation.

Key words: human anterior uvea, lipoxygenase pathway, arachidonic acid, leukotrienes

Acknowledgment. The authors wish to thank Mrs. Ann Zaragoza for typing this manuscript.


References

Direct and Indirect Determination of Nonuniform Cell Density Distribution in Human Corneal Endothelium

B. H. Schimmelpfennig

The density distribution of endothelial cells was determined, directly and indirectly, by counting cells and cell nuclei in two separate groups of unpaired human corneas. Four areas, measuring 1 square mm each, were counted in the corneal center as well as in the periphery close to Schwalbe’s line. In 19 Orcein-stained corneas, the peripheral density of nuclei was 3632/mm² ± 592 (SD) as compared with central counts of 2778 mm² ± 284 (SD). The other group of 22 corneas, stained supravitally with Alizarin-red revealed a peripheral cell density of 3696/mm² ± 721 (SD), in contrast with a central density of 2811/mm² ± 425 (SD). There was also an uneven density distribution in the central endothelium. The average difference between the highest and lowest central square millimeter counts in the two groups was 8.0 ± 7.7%.

No. 2 Reports 223

Direct and Indirect Determination of Nonuniform Cell Density Distribution in Human Corneal Endothelium

B. H. Schimmelpfennig

The density distribution of endothelial cells was determined, directly and indirectly, by counting cells and cell nuclei in two separate groups of unpaired human corneas. Four areas, measuring 1 square mm each, were counted in the corneal center as well as in the periphery close to Schwalbe's line. In 19 Orcein-stained corneas, the peripheral density of nuclei was 3632/mm² ± 592 (SD) as compared with central counts of 2778 mm² ± 284 (SD). The other group of 22 corneas, stained supravitally with Alizarin-red revealed a peripheral cell density of 3696/mm² ± 721 (SD), in contrast with a central density of 2811/mm² ± 425 (SD). There was also an uneven density distribution in the central endothelium. The average difference between the highest and lowest central square millimeter counts in the two groups was 8.0 ± 7.7%.

Acknowledgment. The authors wish to thank Mrs. Ann Zaragoza for typing this manuscript.


Reprint requests: Dr. Prasad S. Kulkarni, PhD, Eye Research Division, Columbia University, 630 West 168th Street, New York, NY 10032.