Selective Binding of a 30-Kilodalton Protein to Disposable Hydrophilic Contact Lenses

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To investigate the accumulation of tear proteins on disposable extended-wear contact lenses (42% Etafilcon A and 58% hydration), a technique involving sodium dodecyl sulfate-polyacrylamide minigel electrophoresis combined with a sensitive silver-staining method was used. Besides the binding of large amounts of tear lysozyme the authors found an accumulation of an as yet unidentified 30-kilodalton (kD) protein. Longitudinal experiments showed lysozyme binding after 1 day of lens wear. The 30-kD protein was detected after a 2-day wearing period. The fact that protein deposition occurs during the relatively short wearing period of these lenses (1 week) may explain the unexpectedly high incidence of contact lens-associated conjunctivitis observed with these lenses. Invest Ophthalmol Vis Sci 31:2244–2247, 1990

Materials and Methods

The lenses used in this study were disposable extended-wear soft contact lenses composed of 42% Etafilcon A (Vistakon, Inc., Jacksonville, FL) and 58% hydration. Disposable contact lenses were worn by healthy individuals, and their lenses were analyzed after obtaining informed consent. Thirty-two lenses were permanently worn by 16 asymptomatic, myopic, cosmetic contact lens wearers for 1–2 weeks. To study the time kinetics by which proteins accumulate on the lens material, a healthy individual was asked to wear the disposable lenses for times of 1 hr to 7 days (1, 2, 4, 8, and 16 hr; 1, 2, 3, 4, 5, 6, and 7 days).

Deposit Solubilization

After removal, each lens was placed in a vial containing 0.9% saline and analyzed immediately or stored at −20°C until use. Lenses were cut with a surgical knife into approximately ten small pieces and placed in a test tube. Subsequently 100 μl of sample buffer (containing 5% sodium dodecyl sulfate [SDS], 2% dithiothreitol, 0.02% bromphenol blue, 2 mM ethylenediaminetetraacetic acid, 0.01% sodium azide, 20 mM Tris HCl, pH 8.0) was added. Samples were heated at 100°C for 20 min and then centrifuged at 12,000 × g for 10 min. This procedure was shown previously to yield optimal solubilization of

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lens deposits. When indicated, samples were concentrated fivefold by centrifugation using an ultrafiltration unit (10-kD cutoff; Millipore, Bedford, MA).

SDS-Polyacrylamide Minigel Electrophoresis and Gel Staining

Samples (1 µl) were applied to commercially available (Pharmacia, Sweden) 10–15% polyacrylamide gradient minigels (43 X 50 X 0.45 mm) and run for 25 min on the Phast System electrophoresis unit (Pharmacia). The protein bands were visualized by an automated highly sensitive silver-staining procedure (1 hr) using the Phast Gel Silver Kit reagents (Pharmacia) according to the manufacturer’s instructions. Besides the contact-lens samples, molecular weight protein standards (Bio-Rad, Richmond, VA), a reference human tear fluid sample, and a sample containing purified human secretory immunoglobulin A (slgA) (Sigma, St. Louis, MO) were run in parallel. Normal human tears were obtained by stimulation with cold air and collected with microcapillaries. Other controls included application of sample buffer after incubation with new, unworn (n = 3) disposable contact lenses.

Protein Identification

Identification of lens deposits was done using peroxidase-labeled monospecific antibodies against human Igs (Dakopatts, Copenhagen, Denmark) or the biotin-labeled lectin concanavalin-A 18 (Con-A; Sigma). Immunologic staining was done after diffusion blotting of the proteins from the polyacrylamide gel onto a 0.45-MITI polyvinyldifluoride membrane (Immobilon, Bedford, MA) at 70°C for 1 hr. The membrane was blocked by exposure to 5% bovine serum albumin phosphate-buffered saline (PBS) containing 0.1% Tween for 30 min at room temperature (RT) and then washed twice (5 min) in PBS with 0.1% Tween. The blot was then incubated with an optimal dilution of the monospecific antibodies for 2 hr at RT. After several washes in PBS-Tween, the membrane was developed using 0.1% dianinobenzide and 0.01% H₂O₂ in PBS for 10 min at RT. Finally the reaction was terminated by adding deionized water to the blot.

Enzymatic lysozyme activity on the lenses was identified using the Micrococcus lysodeicticus enzyme assay 19 after elution in PBS for 24 hr.

Results

Analysis of protein deposits on disposable contact lenses by SDS-polyacrylamide minigel electrophoresis and silver staining revealed an interesting pattern of bands (Fig. 1). The strongest band with a molecular weight of approximately 14 kD represented lysozyme. This could be confirmed in separate experiments using an enzymatic assay for lysozyme. Tear-specific prealbumin and two tear proteins with a lower molecular weight than lysozyme could also be detected (Fig. 1). The following differences became apparent when comparing the lens deposits with the protein pattern of normal tears. The disposable lenses did not appear to bind substantial amounts of the major tear proteins lactoferrin or slgA. On the other hand, a large amount of a protein with a molecular weight of approximately 30 kD could be eluted from the lenses.

The 30-kD band migrated to a similar position as the light chain of slgA present in normal tears (Fig. 1). However, analysis of the 30-kD protein by immunoblotting using antibodies against human Igs revealed that the band was not identical to the lg light chain and probably represented another as yet unknown tear protein (Fig. 2). As a positive control we included slgA and normal human tears in the immunoblotting experiments. As already mentioned above, the lens deposits did not contain detectable slgA heavy chain or the secretory component of slgA.

Analysis of the lens deposits after electrophoresis and staining with the lectin Con-A revealed staining of the 30-kD protein eluted from the lenses (Fig. 3). In normal human tears three prominent bands became apparent of which the upper band coincided with lactoferrin and the secretory component of lgA, the second with the lgA heavy chain, and the lower band stained an unknown protein. Faint staining was also observed with lysozyme. The sample containing slgA showed two bands after Con-A staining which represented the secretory component and the lgA heavy chain.
not detect the 30-kD band on lenses worn between 1 hr and 1 day. The 30-kD band became detectable on lenses worn for 2 days and deposition remained constant up to day 7 (Fig. 4).

Discussion

Our results show the binding of a 30-kD tear protein to disposable contact lenses made of Etafilcon A material. The introduction of SDS-polyacrylamide minigel electrophoresis combined with sensitive silver-staining methods has opened new perspectives to analyze the interaction of tear constituents and contact lens materials. Current methods to analyze tear-protein deposits on contact lenses have involved laborious and time-consuming procedures. Slab-gel electrophoresis of tear proteins takes 2 days to obtain the final results whereas the automated minigel electrophoresis technique described here is finished in 1 hr. Furthermore silver staining is highly sensitive and can detect proteins almost tenfold better than Coomassie blue stain.

The 30-kD protein eluted from the lens material could have represented a selective binding of the light chain of slgA, which has a similar molecular weight. Various arguments exclude this explanation. First of all the 30-kD protein eluted from the lenses did not react with Ig antibodies. Furthermore the 30-kD band reacted with Con-A, and the slgA light chain did not. The contact lenses did not contain the other subunits of slgA such as the heavy chain or the secretory component, which also makes it unlikely that the 30-kD band was identical to the Ig light chain.

The 30-kD protein is also not identical to the 31-kD protein described by Gachon et al since that protein dissociates in the presence of disulfide band-reducing agents. The sample buffer used in our study...
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