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

Kam H. Cheng,* Jan H. C. Kok,* Cor van Mil,* and Aize Kijlstra†

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-kilo-dalton (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

Daily-wear contact lenses require frequent removal for cleaning and disinfection. This exposes the patient to potentially irritating components of the cleaning solutions, which may lead to allergic kerato-conjunctivitis.1 Microbial keratitis possibly due to improper lens cleaning, personal hygiene habits, or contamination of contact lens care systems2 is a well-known potentially sight-threatening complication of contact lens use. Increased risk of ulcerative keratitis with reusable extended-wear soft contact lenses has been reported.3-6

Another well-recognized disorder associated with contact lens wear is contact lens-induced giant papillary conjunctivitis. Protein accumulation on lenses has been implicated as a possible etiology for giant papillary conjunctivitis.7 Deposit formation on soft contact lenses has been studied extensively using various techniques, including electrophoretic procedures,8-11 standard analytic chemical assays,8,12 histo-chemical staining,9,10 electron microscopy,12-14 immunofluorescence microscopy,8,13 and binding of radiolabeled artificial tear proteins.16

The introduction of disposable extended-wear contact lenses may open new alternatives for eliminating the spectrum of adverse effects associated with the use of daily-wear and reusable extended-wear contact lenses.17 It is possible that accumulation of tear proteins will not occur during the relatively short wearing period of a disposable contact lens. To investigate this hypothesis we used a sensitive technique to analyze the protein deposits on disposable contact lenses. Apart from the already known uptake of tear lysozyme on hydrophilic lenses,15 we found a strong accumulation of an as yet unidentified protein with an apparent molecular weight of 30 kilodaltons (kD).

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
lens deposits.\(^8\) 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 \(\mu \text{l}\)) 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 (sIgA) (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 IgGs (Dakopatts, Copenhagen, Denmark) or the biotin-labeled lectin concanavalin-A (Con-A; Sigma). Immunologic staining was done after diffusion blotting of the proteins from the polyacrylamide gel onto a 0.45-\(\mu\text{m}\) polyvinylidifluoride 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% diaminobenzidine and 0.01% \(\text{H}_2\text{O}_2\) 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\(^9\) 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 sIgA. 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 sIgA 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 Ig light chain and probably represented another as yet unknown tear protein (Fig. 2). As a positive control we included sIgA and normal human tears in the immunoblotting experiments. As already mentioned above, the lens deposits did not contain detectable sIgA heavy chain or the secretory component of sIgA.

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 IgA, the second with the IgA heavy chain, and the lower band stained an unknown protein. Faint staining was also observed with lysozyme. The sample containing sIgA showed two bands after Con-A staining which represented the secretory component and the IgA heavy chain.
MW (kD) 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 1hr/2 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 IgA, 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 IgA light chain did not. The contact lenses did not contain the other subunits of IgA 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

---

Fig. 2. Analysis of protein deposits by immunoblotting on disposable extended-wear contact lens (wearing period 2 days) using peroxidase-labeled antibodies against human immunoglobulins. Samples applied were normal human tears (lane 1), purified human colostrum secretory IgA (lane 2), fivefold concentration of contact lens eluate (lane 3), and original contact lens eluate (lane 4).

Fig. 3. Lectin Con-A staining after SDS-polyacrylamide minigel electrophoresis and diffusion blotting of lens deposits to polyvinylidene difluoride membrane. Samples applied were normal human tears (lane 1), purified human colostrum secretory IgA (lane 2), concentrated contact lens eluate (lane 3), and original contact lens eluate (lane 4).

Fig. 4. Analysis of the time course of protein deposits on disposable extended-wear contact lenses as assessed by SDS-polyacrylamide minigel electrophoresis and silver staining. Lanes 1–3 represent contact lenses worn for 1, 2, and 7 days, respectively. Lane 4 is a sample containing human tears.
contained dithiothreitol as a reducing agent and caused dissociation of multichain proteins such as slgA or the Gachon protein into separate polypeptide chains.

The origin of the 30-kD protein and its concentration in tears is not yet known. Incubation of a new lens with reflex human tears in vitro during 5 days at 37 °C with four times refreshment of tears also resulted in the appearance of the 30-kD protein on the lens. This indicates that the protein is present in the tears but does not yet show where it originates.

The identity or function of the observed protein is not yet known. The fact that it accumulates on the ionic lens material and binds Con-A may provide clues to its further characterization. The lectin Con-A has been used previously to identify glycoprotein deposits on hydrogel contact lenses. It may be possible that the 30-kD protein is one of the soluble mucous glycoproteins that exist in tears. Our data show that a detectable buildup of the 30-kD protein occurs even after short wearing periods of these disposable extended-wear soft contact lenses. This finding may explain the unexpectedly high incidence of contact lens-induced conjunctivitis with the Etafilcon A disposable lenses.

Key words: SDS-polyacrylamide gel electrophoresis, contact lenses, protein deposits, tear proteins

References