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Accelerated study on lysozyme deposition on poly(HEMA) contact lenses

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Abstract

A technique was developed to accelerate lysozyme deposition on poly(HEMA) contact lenses and measure the amounts of the deposited lysozyme. This technique was for evaluation of bendazac lysine solution, a contact lens cleaning and wetting solution. Effect of temperature on lysozyme deposition on poly(HEMA) contact lenses was examined. Five temperatures ranging from 25°C to 90°C were chosen to examine the temperature effect. The amounts of lysozyme deposited on poly(HEMA) contact lenses at 25°C and 60°C were 0.27 μg/lens and 0.61 μg/lens, respectively. The amount increased sharply to 23 μg/lens at 70°C with the maximum of 31 μg/lens at 90°C. Kinetics of lysozyme deposition on poly(HEMA) contact lenses was examined at 80°C. Lysozyme deposition increased sharply during the first 2 h and reached a plateau after 2 h. Effectiveness of various cleaning procedures was examined using bendazac lysine solution. When the contact lenses were washed without rubbing with fingers, the bendazac lysine reduced the amount of deposited lysozyme by more than 40% from 18.3 μg/lens to 10.6 μg/lens. The effect of bendazac lysine was most prominent when the contact lenses were shaken during storage in the presence of lysozyme in solution. If the contact lenses were cleaned by rubbing with fingers, the effect of bendazac lysine solution on the prevention of lysozyme deposition was negligible.

Keywords: Contact lens, Poly(HEMA), lysozyme, accelerated deposition, bendazac lysine

1. Introduction

It has been estimated that 80% of all clinical problems occurring in soft contact lens wearers are traceable to surface spoilage resulting from accumulation of tear fluid components onto the contact lens [1]. One of the many factors that predispose the lens to deposit formation is improper lens care [1]. Contact lenses accumulate proteins, lipids and other components of tear fluid on their surface as well as in their matrix [2,3]. This accumulation is suspected of contributing to microbial contamination leading to infection, allergic and inflammatory reactions [4], and mechanical irritation all with serious consequences [5]. Development of giant papillary conjunctivitis in the approximately 15% of the soft contact lens wearers is attributed to mechanical irritation due to the deposit on the soft contact lenses, and it usually responds to meticulous cleaning of the contact lens [1,6]. It is often necessary for contact lens users to clean contact lenses after each use. The nature and composition of deposit vary but it is a combination of mucous, proteins, lipids and debris [6–9]. Experiments have shown that the major protein components of the deposit are lysozyme, albumin, and gamma-globulin with lysozyme being the most abundant component [10]. This is due to the fact that lysozyme shows greater affinity for the polymers of contact lens than the other proteins [11]. In addition, lysozyme accounts for 30% of the total proteins in tear fluid [4].

Prevention of lysozyme deposition is important in keeping contact lenses clean and avoiding problems associated with contact lenses mentioned above. The study on the effect of contact lens cleaning solution on the prevention of lysozyme deposition is time-consuming, since lysozyme deposition is a slow process. The efficient methods for studying lysozyme deposition and its prevention onto contact lenses require in vitro models which

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accelerate spoilage of contact lenses and a sensitive method for quantification of the deposited lysozyme. Lysozyme has been heated at 100°C to induce the in vitro formation of lysozyme deposits on contact lenses [2]. We examined the effect of temperature on lysozyme deposit onto poly(hydroxyethyl methacrylate) (poly(HEMA)) contact lenses. The amount of the deposited lysozyme was quantitated by using ^{125}I-labeled lysozyme. The effect of various cleaning procedures was also examined using bendazac lysine solution.

2. Materials and methods

2.1. Radio-iodination of lysozyme

Chicken egg lysozyme (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS, pH 7.4) was radio-labeled with ^{125}I using the Enzymo-Bead® Reagent (Bio-Rad, Hercules, CA). The radio-labeled protein was separated from free ^{125}I by gel filtration over a column packed with Bio-Gel P6-DG (Bio-Rad, exclusion limit of 6,000) which was equilibrated with PBS and calibrated with blue dextran (MW of 2,000,000, Sigma, St. Louis, MO). Radio-labeled lysozyme was mixed with the native protein in a weight ratio of 1 : 39.

2.2. Effect of temperature

Five temperatures ranging from room temperature to 90°C were chosen to examine the effect of temperature on lysozyme deposition on poly(HEMA) contact lenses (Hydron®, Ocular Sciences, San Francisco, CA). Poly (HEMA) contact lenses previously hydrated with PBS were placed individually in a test tube and 4 ml of lysozyme solution at the concentration of 0.4 mg ml^{-1} in PBS was added to the tube. The test tubes were sealed and placed in a thermostat-controlled water bath at a particular temperature for 4 h. They were then allowed to cool to room temperature for 1 h and subsequently rinsed with three 10 ml portions of PBS. After lenses were blotted to remove excess aqueous solution, the radioactivity on each contact lens was counted using a gamma counter (Gamma 5500B, Beckman, Arlington Height, IL). A total of six samples from three independent experiments were used to calculate the amount of lysozyme deposited on the lenses at each temperature.

2.3. Kinetics of lysozyme deposition

The effect of exposure time on the lysozyme deposition was examined at 80°C. Contact lenses were exposed to lysozyme solution for various periods of time at 80°C, and the amounts of lysozyme deposited onto poly(HEMA) contact lenses were calculated as described above.

Table 1
Four treatment groups of test and control lenses

Group	Treatment
I	six test and 6 control lenses were removed and rinsed with PBS
II	six control and 6 test lenses in respective solutions were shaken on a mechanical shaker (American rotator V, American Rotors Inc. Gurnee, IL) at a speed of 100 movements min^{-1} for 3 min
III	six control and 6 test lenses were individually rubbed in the palm of a gloved hand
IV	six control and 6 test contact lenses were shaken using a mechanical shaker (American rotator V) at a speed of 100 movements min^{-1} for 3 min to obtain a mechanical, reproducible cleaning. All lenses were then individually rubbed in the palm of a gloved hand

2.4. Effect of bendazac lysine solution

The poly(HEMA) contact lenses were cleaned with bendazac lysine solution which is commercially available for wetting and cleaning of soft contact lenses [2]. Bendazac lysine, a nonsteroidal anti-inflammatory drug (NSAID), is a lysine salt of [(1-(benzyl-1H-indazole-3-yl)oxy] acetic acid. The compound has a molecular weight of 428 Da and is a crystalline powder with a melting point of 177 ~ 193°C [12]. It is soluble in water, poorly soluble in ethanol, and almost insoluble in chloroform, ether, and octanol. The bendazac lysine solution used in this study is a 0.25% (w/v) solution in a borate buffer (pH 7.2), which is available commercially as 3-in-1® drop (Angelini Pharmaceuticals Inc., River Edge, NJ).

Forty-eight contact lenses were equally divided into two groups of control and test samples. Each of the 24 control lenses was placed in a test tube containing 4 ml of lysozyme solution (0.4 mg ml^{-1}) in PBS and kept at 80°C for 4 h. All of the test lenses were soaked in bendazac lysine solution for 5 min. Then, each contact lens was placed in a test tube containing 4 ml of lysozyme (0.4 mg ml^{-1}) in 0.25% (w/v) bendazac lysine solution and kept at 80°C for 4 h. The tubes were cooled to room temperature for 1 h.

Test and control lenses were divided into 4 treatment groups with each treatment group containing six control and six test lenses (Table 1). Group I was the control group where no cleaning of contact lenses, other than rinsing with PBS, was involved. Lenses in Groups II, III and IV were subjected to various cleaning procedures simulating the procedures routinely used by contact lens wearers to clean contact lenses. After cleaning, all the lenses were rinsed with three 10 ml portions of PBS and blotted to remove excess water. The deposited lysozyme was determined by measuring the radioactivity using a gamma counter.

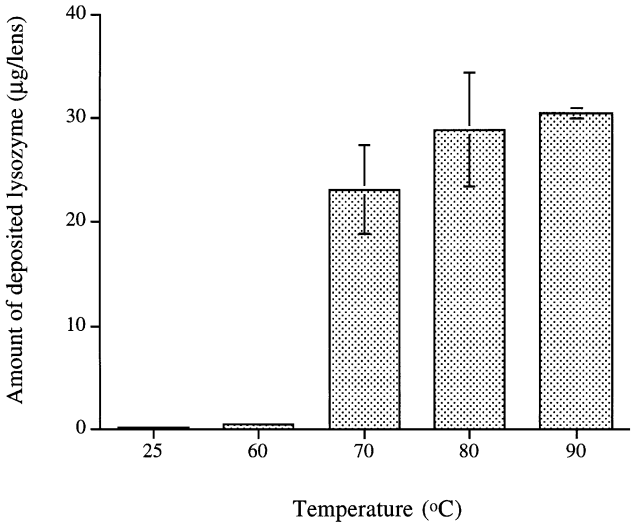

Fig. 1. Effect of temperature on the amount of lysozyme deposited on poly(HEMA) contact lenses. Contact lenses were exposed to the lysozyme solution for 4 h. Average ± S.D. $n = 3$.

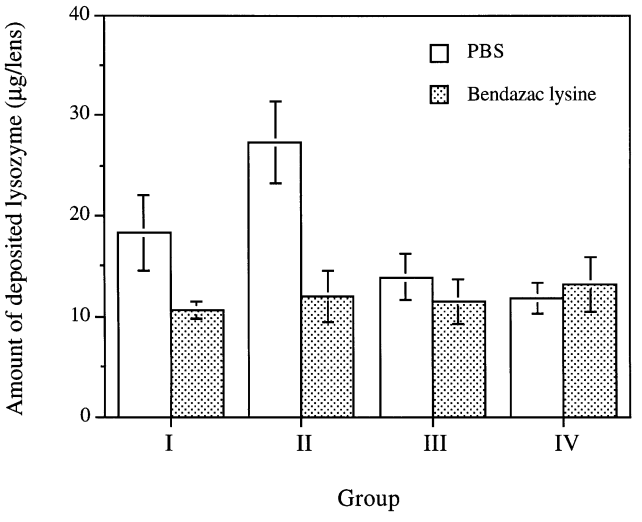

Fig. 3. The amount of lysozyme deposited on the contact lenses in PBS and in bendazac lysine solution in Groups I–IV. Contact lenses were exposed to the lysozyme solution for 4 h at 80°C. Average ± S.D. $n = 6$.

3. Results

Five representative temperatures in the range of 25–90°C were selected to examine the effect of temperature on the deposition of lysozyme on poly(HEMA) contact lenses. Fig. 1 shows that lysozyme deposition onto poly(HEMA) contact lenses is minimal at 60°C and lower. The amount of lysozyme deposited onto the contact lenses at 25 and 60°C were 0.27 and 0.61 μg lens^{-1}, respectively. The amount of lysozyme deposited onto contact lenses increased sharply to 23 μg lens^{-1} at 70°C. The lysozyme adsorption reached the maximum to 31 μg lens^{-1} at 90°C. Since the lysozyme adsorption at 80°C was statistically indifferent from that at 90°C ($p < 0.05$), we chose 80°C for the accelerated lysozyme deposition for subsequent studies.

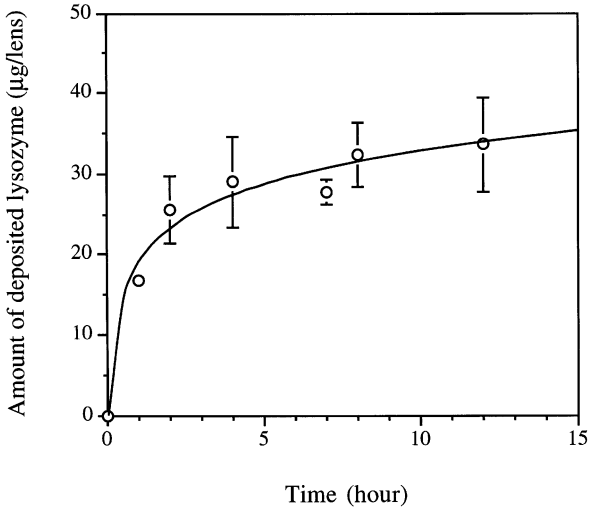

Fig. 2. Effect of duration of contact lenses exposure to the lysozyme solution on the amount of lysozyme deposited on the lenses at 80°C. Average ± S.D. $n = 3$.

The kinetics of lysozyme deposition at 80°C was examined to find out the time to reach the plateau of lysozyme deposition. As shown in Fig. 2, lysozyme deposition increased sharply during the first 2 h of deposition and then slowed down. A steady increase in lysozyme deposition was observed during the 2–12 h deposition time period. Although, the increase in deposition time seemed to increase the amount of deposited lysozyme, the increase was not substantial. For this reason, we chose 4 h of lysozyme deposition at 80°C for later studies on the effect of contact lens cleaning procedures.

The effectiveness of various cleaning procedures in preventing deposition of lysozyme on poly(HEMA) contact lenses was examined using bendazac lysine solution. The effectiveness of wetting and cleaning solutions for contact lenses depends on how well they prevent or remove the deposited components of tear fluid such as lysozyme. Four cleaning procedures with and without bendazac lysine solution were investigated. Fig. 3 shows the amount of lysozyme deposited on contact lenses subjected to different cleaning procedures. When the contact lenses were washed without cleaning by rubbing with fingers, the bendazac lysine solution reduced the amount of deposited lysozyme by more than 40% from 18.3 to 10.6 μg lens^{-1} (Group I). The effect of bendazac lysine solution was most prominent when the contact lenses were shaken during storage in the presence of lysozyme in solution (Group II). While the shaking process increased the amount of deposited lysozyme in

the control group, it did not affect the lysozyme deposition in the bendazac lysine solution. There was no significant difference in lysozyme deposition for Groups III and IV, which involved individual rubbing of contact lenses in the palm of a gloved hand. It appears that rubbing of contact lenses effectively remove much of the deposited lysozyme. The rubbing, however, did not remove all the deposited lysozyme. Approximately 12 μg of lysozyme still remained on each contact lens even after individual rubbing. The effectiveness of bendazac lysine solution in Groups I and II suggests that the use of contact lens wetting and cleaning solutions is effective for prevention of lysozyme deposition, since it is highly likely that the contact lens users skip hand cleaning of their contact lenses.

4. Discussion

The in vitro deposition of lysozyme on soft contact lenses is usually examined by incubating the contact lens with lysozyme solution in buffer or artificial tear fluid at 37°C for an extended period of time [13,14]. Lysozyme deposition on soft contact lenses is a rather slow process and takes long time to give a quantitative amount of lysozyme at this temperature. Hence, these procedures take days and even months to complete. In addition, the amount of lysozyme deposited under such conditions is low. While these methods produce contact lens spoilage equivalent to several months of normal wear, it is desirable if an accelerated test can be done in a much shorter period of time, e.g. less than 24 h. Heat denaturation method involves heating up the lysozyme solution in the presence of the contact lens in order to get high deposition. Heat denaturation at 100°C was done but the effect of temperatures and time of exposure have not been examined systematically [2]. We examined the effect of temperature to find milder condition for the heat denaturation method. We found that lysozyme deposition at 80°C for 4 h is equivalent to that at 100°C for 1 h found in the literature [2].

The amounts of lysozyme deposited onto poly(HEMA) contact lenses at temperatures 70°C and above were much larger than the monolayer coverage as shown in Fig. 1. Assuming the nominal surface area of 2.65 cm^2 for each contact lens, the monolayer lysozyme coverage is expected to be 0.3 $\mu g\,cm^{-2}$, since lysozyme is 31 Å in diameter and has a molecular weight of 14,500 Da. These high lysozyme concentrations on contact lenses indicate a multi-layer and/or aggregate deposition on the surface as well as inside matrix of the lenses. Approximately 40-fold increase in lysozyme deposition by change in temperature from 60 to 70°C indicates that lysozyme denaturation becomes extensive at 70°C. It is not clear at this point, how such a denaturation increases deposition onto the contact lenses, but such a process provides a means to study lysozyme deposition in accelerated studies.

The deposition of lysozyme on soft contact lenses has been investigated using a variety of techniques such as Coomasie blue staining [15], ESCA [16], FTIR/ATR [17,18], amino acid analysis [4,19], fluorescence [9], immunochemical [20], spectroscopic [21] and colorimetric [2] methods. These techniques are either too complicated or are inaccurate in predicting the nature and amount of lysozyme deposition on contact lenses. Measurement of UV absorbance by the contact lens is relatively simple but the accuracy of this method is questionable due to high background UV absorbance by the contact lens itself. Use of radio-tracers such as ^{14}C- or ^{125}I-labeled lysozyme [14,22] present a very accurate and reliable quantitative technique for assessing protein deposition on contact lenses. A simple and yet accurate protein detection technique is essential to be able to quickly evaluate the effectiveness of contact lens cleaning and wetting solutions. In this study, we used ^{125}I-labeled lysozyme to quantify the amount of lysozyme deposited onto poly(HEMA) contact lenses, and as shown in Figs. 1 and 2, the information obtained is highly quantitative and reliable.

Enzymatic and non-enzymatic solutions are routinely used to clean soft contact lenses. Non-enzymatic cleaning solutions typically contain a non-ionic surfactant, a wetting agent, a chelating agent, buffers and preservatives. Some cleaning solutions contain mild abrasives (e.g. Opti-Clean II®, Alcon Inc., Fort Worth, TX) to aid the cleaning process by physically removing the deposited debris, while others contain isopropyl alcohol (e.g. Miraflow®, Ciba Vision, Duluth, GA) to help remove lipid deposits. Non-enzymatic cleaning solutions are quite effective in removing lipid deposits but are ineffective against tenacious protein debris. Enzymatic solutions contain enzymes capable of digesting the protein debris and are mainly used to remove protein buildup on soft contact lenses. Bendazac lysine solution is a non-enzymatic cleaning solution which is used for wetting and cleaning of soft contact lenses. A few drops of this solution is applied to a contact lens, which is then gently rubbed between the fingers for about 15 s and subsequently, rinsed with appropriate rinsing solution before use or storage [23]. As shown in Fig. 3, the appropriate use of such a solution is effective in decreasing lysozyme deposition by 56%. It is noted that the experimental condition used in our study (i.e., lysozyme adsorption in the presence of bendazac lysine) did not represent actual adsorption of lysozyme onto contact lenses, since there would be no continuous supply of bendazac lysine during contact lens wearing. However, a recent clinical evaluation of bendazac lysine solution in vivo showed the effectiveness of this solution in reducing the amount of protein deposition on soft contact lenses [24]. Thus, it appears that bendazac lysine solution is another useful solution for cleaning and wetting contact lenses.

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