Fluorophotometric measurement of the precorneal residence time of topically applied hyaluronic acid

H Mochizuki,1 M Yamada,1 S Hato,1 T Nishida2

ABSTRACT

Purpose: This study was performed to separately assess the aqueous flow applied with hyaluronic acid, and the behaviour of hyaluronic acid itself on the ocular surface.

Methods: Two different fluorescent dyes, fluorescein sodium dissolved in 0.1% hyaluronic acid (HA) solution and 0.1% fluorescein conjugated with hyaluronic acid (F-HA) dissolved in saline, were used. A volume of 20 μl of tested solution was applied to the eye of 10 healthy volunteers. Fluorescein sodium dissolved in saline served as a control. The fluorescent intensity of the precorneal tear film was measured at the central cornea every minute for 10 min. The turnover rate was calculated using the equation that plots fluorescent intensity against time in a semilog plot and expressed as %/min.

Results: Turnover rates of topically applied 0.1% F-HA, 0.1% HA and saline were 8.1 (SD 3.6)%/min, 21.6 (2.8)%/min, and 31.0 (3.7)%/min, respectively. The turnover rate of F-HA was significantly lower than that of HA and saline (p = 0.00012 and p = 0.00000022, respectively; Mann–Whitney test). The turnover rate of HA was significantly lower than that of saline (p = 0.00001; Mann–Whitney test).

Conclusion: Our results indicate that the bulk aqueous flow applied with HA and the turnover of HA itself are different. HA molecules may adhere to the ocular surface by surface-chemical and/or biochemical properties. The long retention time of HA on the ocular surface may explain the mechanism in which hyaluronic acid has been shown to enhance tear film stability for a few hours.

Dry eye is a common condition, affecting approximately 10–20% of the adult population.1 The clinical consequences of dry eye may include symptomatic irritation, superficial punctuate keratoatyphy, corneal erosions and possibly visual acuity problems.2 Dry eye is considered to be a disorder of the tear film due to tear deficiency or excessive evaporation, which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort.3

A variety of treatment modalities have been used for the treatment of dry eye. The majority of these fall into the category of tear substitutes or replacements.4 Artificial tears, the most frequently used modality for the treatment of dry eye, may be effective in relieving symptoms in mild dry eye by replenishing deficient tear volume. However, in moderate and severe cases of dry eye, artificial tears alone are not enough to relieve the symptoms nor to improve superficial punctate keratoatyphy.1

Since preliminary reports in the early 1980s,5–7 several studies have reported that hyaluronic acid is able to improve the symptoms, signs and ocular surface damage associated with dry eye syndrome.8–11 Hyaluronic acid is a glycosaminoglycan with a viscoelastic rheology. Its relatively high viscosity is believed to improve tear-film stability and to reduce washout from the ocular surface.12 Hyaluronic acid enhances water retention on the corneal surface, and probably increases corneal wettability.13 In addition, hyaluronic acid promotes migration of corneal epithelial cells and accelerates the healing of corneal epithelial defects.14–16 Hyaluronic acid has thus become an important treatment modality for dry eye.

Conflicting results, however, have been obtained regarding the duration of the action of hyaluronic acid on the ocular surface. The residence time of topically applied hyaluronic acid assessed by the tear meniscus height and water evaporation rate from the ocular surface was less than 10 min, although this was significantly longer than that of phosphate-buffered saline.17–18 Using an assessment of the tear-film breakup time, however, hyaluronic acid has been shown to enhance tear-film stability for more than a few hours.19–21 These observations suggest that hyaluronic acid remains on the ocular surface independent of the bulk aqueous flow.

In order to test this hypothesis, we used two different fluorescent dyes, fluorescein sodium dissolved in hyaluronic acid solution and fluorescein conjugated with hyaluronic acid dissolved in saline, in the current study. The former is a well-established dye used to assess the bulk aqueous flow, and the latter dye is used as a tracer to determine the behaviour of hyaluronic acid on the ocular surface. Although the residence time of topically applied hyaluronic acid has been investigated using 99mTc as a tracer,22–24 we believe that this study is the first report to measure the residence time using a tracer that is associated with hyaluronic acid on the ocular surface.

SUBJECTS AND METHODS

Fluorescent dye and fluorophotometer

Fluorescein hyaluronic acid (F-HA, Mw; 800 000 Da) was purchased from Sigma-Aldrich (St. Louis, MO). F-HA, fluorescein conjugated with hyaluronic acid, was dissolved in a phosphate-buffered saline as 0.1% solution and used as a tracer of hyaluronic acid. Fluorescein sodium (0.001%; Sigma-Aldrich) and 0.1% hyaluronic acid (Mw; 800 000 Da, Sigma-Aldrich) dissolved in a phosphate-buffered saline was used as a tracer of the bulk aqueous flow in the presence of hyaluronic acid. Fluorescein sodium (0.001%) in a phosphate-buffered saline was used as a tracer of the bulk aqueous flow.
RESULTS

Calibration of F-HA
The calibration of the fluorescent intensities against the concentrations of 0.001–0.5% F-HA is shown in fig 1. The relationship between the fluorescent intensities and the concentrations of F-HA was linear ($r^2 = 0.995$). The data generated by this method were consistent and reproducible. The fluorescent intensities of F-HA were unaffected by the presence of 1% fetal bovine serum (data not shown).

Turnover-rate measurements
A typical result of turnover-rate measurements obtained from one subject is shown in fig 2. In the presented case, the fluorescent intensities of 0.1% F-HA decayed with time at a flow rate of 7.6%/min, which was lower than those of fluorescein sodium in 0.1% hyaluronic acid (19.4%/min) and in saline (28.1%/min).

The turnover rates of topically applied F-HA, hyaluronic acid and saline obtained from 10 subjects were 8.1 (3.6)%/min, 21.6 (2.8)%/min, and 31.0 (3.7)%/min, respectively (table 1). The turnover rate of F-HA was significantly lower than those of hyaluronic acid and saline ($p = 0.00012$, and $p = 0.00000022$, respectively; Mann–Whitney test). The turnover rate of F-HA was significantly lower than that of saline ($p = 0.00001$; Mann–Whitney test).

DISCUSSION

In the current study, we used two different fluorescent dyes, fluorescein sodium dissolved in hyaluronic acid solution and F-HA solution, to separately assess the aqueous flow applied with hyaluronic acid, and the behaviour of hyaluronic acid itself on the ocular surface. Our results indicate that there are two different aspects of the duration of topically applied hyaluronic acid.

Hyaluronic acid has a high-molecular-weight, naturally occurring glycosaminoglycan. Its relatively high viscosity is believed to reduce washout from the ocular surface. The residence time of topically applied hyaluronic acid has previously been investigated by using gamma scintigraphic methods. Snibson and associates reported that hyaluronic acid had prolonged ocular residence times in comparison

Figure 1  Calibration of the fluorescent intensities against the concentrations of fluorescein hyaluronic acid (F-HA). The relationship between the fluorescent intensities and the concentrations of F-HA was linear ($r^2 = 0.999$).

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with a buffered saline solution, a solution containing polyvinyl alcohol or hydroxypropylmethylcellulose. In the current study, the turnover rate of the 0.1% hyaluronic acid solution (21.6 (2.3)%/min) was significantly lower than that of the saline (31.0 (3.7)%/min). Our result is considered to be in good accordance with previous studies using scintigrafic methods.22–24 This effect, however, appears to be transient, because 90% of the hyaluronic acid solution was calculated to be cleared from the ocular surface 10.7 min after instillation. This result is also consistent with the duration of topically applied hyaluronic acid assessed by the tear meniscus height and water evaporation rate from the ocular surface.17 18

The most interesting finding of the current study is that the turnover rate of F-HA (8.1 (3.6)%/min) was approximately one-third of the 0.1% hyaluronic acid solution (21.6 (2.3)%/min). This result indicates that the bulk aqueous flow applied with hyaluronic acid and the turnover of hyaluronic acid itself on the ocular surface are different. Besides viscosity, hyaluronic acid molecules may adhere to the ocular surface by surface-chemical and/or biochemical properties, because hyaluronic acid is known to bind with fibronectin and CD44, a cell surface adhesion molecule which has been found on corneal epithelial cells.16 26 Snibson and associates24 made a similar consideration based on their scintigrafic results. They, however, also mentioned the limitation of their methodology and the necessity of tracers that directly associate with hyaluronic acid. We believe that F-HA is a useful tracer to determine the behaviour of topically applied hyaluronic acid. According to our results, the times for 90% and 99% of hyaluronic acid to be cleared from the ocular surface were calculated to be 28.3 min and 57.6 min after instillation, respectively. The long retention time of hyaluronic acid in the ocular surface may explain the fact that hyaluronic acid has been shown to enhance tear-film stability for more than a few hours.10–21

Besides its biological effects on the corneal epithelial cells, hyaluronic acid appears to have two beneficial effects for the treatment of dry eye syndrome. First, it reduces the bulk aqueous flow by its viscosity and increases tear volume for a limited time, as do other viscous agents, such as chondroitin sulfate, polyvinyl alcohol and hydroxypropylmethylcellulose.25 Second, hyaluronic acid remains on the ocular surface for a longer time, in order to increase corneal wettability and to retain tear fluid on the corneal surface.12 13 This effect may be unique for hyaluronic acid, although it should be confirmed by further investigations.

Competing interests: The authors have no proprietary interest in any materials in this manuscript.

REFERENCES


Table 1  Turnover rates of topically applied 0.1% fluorescein hyaluronic acid (F-HA), 0.1% hyaluronic acid and saline obtained from 10 subjects

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>F-HA (SD)</th>
<th>Hyaluronic acid (SD)</th>
<th>Saline (SD)</th>
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<tbody>
<tr>
<td>1</td>
<td>7.6 (3.6)</td>
<td>19.4 (3.6)</td>
<td>28.1 (3.7)</td>
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<td>2</td>
<td>12.2 (2.8)</td>
<td>22.6 (2.8)</td>
<td>31.0 (3.7)</td>
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<tr>
<td>3</td>
<td>2.9 (4.6)</td>
<td>22.6 (2.8)</td>
<td>31.0 (3.7)</td>
</tr>
<tr>
<td>4</td>
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<td>33.2 (3.8)</td>
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<tr>
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<tr>
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<tr>
<td>10</td>
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<td>21.3 (3.8)</td>
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<tr>
<td>Mean (SD)</td>
<td>8.1 (3.6)</td>
<td>21.7 (3.8)</td>
<td>31.0 (3.7)</td>
</tr>
</tbody>
</table>

The turnover rate of F-HA was significantly lower than those of hyaluronic acid and saline (p = 0.00012 and p = 0.00000022, respectively; Mann–Whitney test).

Figure 2  Typical result of the turnover rate measurements obtained from one subject. In the presented case, the fluorescent intensities of 0.1% fluorescein hyaluronic acid (F-HA) decayed with time at a flow rate of 7.6%/min, which was lower than those of fluorescein sodium in 0.1% hyaluronic acid (19.4%/min) and in saline (28.1%/min).

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