Effects of Acetylcysteine on Rabbit Conjunctival and Corneal Surfaces

A Scanning Electron Microscopy Study

Florence Thermes, Sylvain Molon-Noblot, and Jeffrey Grove

Conjunctival and corneal epithelial surfaces of normal rabbit eyes with their associated mucus were studied by scanning electron microscopy before and after treatment with the mucolytic agent N-acetylcysteine (AC). Four groups received topically one 50-μl drop of either (Group A) 0.1 M AC, (Group B) 0.1 M AC every 5 min for 1 hr, (Group C) 0.1 M AC every 5 min for 2 hr, or (Group D) three drops of 20% AC over 15 min. The effects of the instillation of AC on mucus removal and cellular lesions increased in the order (A) < (B) < (C) < (D). Treatment A had no effect on cornea and conjunctiva. Treatment B cleaned away mucosal debris without alteration of either conjunctival or corneal epithelium. Treatment C had a similar effect on the mucus but was associated with focal necrosis, and treatment D produced widespread necrosis, desquamation of epithelial cells, and inflammation. Invest Ophthalmol Vis Sci 32:2958-2963, 1991

N-Acetylcysteine (AC) was first used by Webb1 as a mucolytic agent for the liquefaction of tenacious bronchial secretions. Its efficiency was confirmed in another study that found AC to be the least irritating and the most stable among several sulfhydryl agents.2 Because of its mucolytic and anticollagenolytic properties, AC has been used in ophthalmology to treat corneal diseases,3,4 such as keratoconjunctivitis sicca,3 filamentous keratitis,5 and corneal catarrh, and alkali-burned corneas.6,7

Corneal toxicity of AC was observed after intracorneal injection,8 and evaluation of its effects on the cornea and mucus was made using light microscopy9,10 and histochemistry.11 However, a detailed morphologic examination of the effect of AC on the ocular surfaces was not done to our knowledge. Although different treatments with AC were described,9,12,13 the effective dose without undue toxicity has not been established. We therefore examined rabbit conjunctival mucosa and cornea using scanning electron microscopy (SEM) after topical application of various solutions of AC currently recommended.

Materials and Methods

Materials

We purchased AC from Sigma (La Verpillière, France), and solutions were prepared fresh on the day of the experiment. The pH was adjusted to 8 with 0.1 M NaOH.14 All other chemicals were analytic grade and obtained commercially.

New Zealand rabbits were supplied by Charles River (France). They were housed individually with a 12-hr light-12-hr dark cycle and had free access to food and water. Our investigations conformed to the ARVO Resolution on the Use of Animals in Research.

AC Instillation

The rabbits were placed in wooden restraining boxes to minimize movement. Unilaterally, we instilled 50-μl drops of AC into the left conjunctival sac, and the lower lid was brought gently up to meet the upper. The contralateral eye was not treated and served as the control.

Three rabbits were used for each of the treatment groups reported in Table 1. Macroscopic examination of corneal and conjunctival surfaces was done on all rabbits before sampling.

The animals were killed by rapid injection into the marginal ear vein of a lethal dose of sodium pentobarbital. Biopsy specimens were shaved off the lower pal-
Table 1. Protocol design for topical application of N-acetylcysteine in rabbit left eyes (untreated right eyes were used as controls)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC concentration</td>
<td>0.1 M</td>
<td>0.1 M</td>
<td>0.1 M</td>
<td>1.23 M (20%)</td>
</tr>
<tr>
<td>Volume instilled</td>
<td>50 μl</td>
<td>50 μl</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
<tr>
<td>No. of instillations</td>
<td>1</td>
<td>12</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Intervals between instillations</td>
<td>—</td>
<td>5 min</td>
<td>5 min</td>
<td>5 min</td>
</tr>
<tr>
<td>Sacrifice</td>
<td>30 min</td>
<td>1 hr</td>
<td>2 hr</td>
<td>15 min</td>
</tr>
</tbody>
</table>

pebral conjunctiva (5 × 5 mm) and off the cornea (3 × 3 mm). During the processing of samples, care was taken not to touch the specimens at any time before the SEM examination.

SEM

Biopsy specimens were fixed by immersion in a 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C for 2 hr. They were rinsed rapidly in 0.2 M cacodylate buffer and then fixed in a 1% osmium tetroxide solution in 0.1 M cacodylate buffer for 1 hr at room temperature.

The specimens were dehydrated in a series of graded alcohol solutions, immersed in amyl acetate, and subsequently dried in liquid carbon dioxide using a critical-point drying apparatus (CPD010; Balzers Union, Liechtenstein). They were mounted with silver-conducting paint on aluminum stubs and coated with gold and palladium in a sputtering apparatus (SCD030; Balzers) under a residual argon atmosphere. We used a SEM (JSM T200; Jeol, Tokyo, Japan) under an accelerating voltage of 25 kev.

To evaluate the effect of AC in quantitatively, each biopsy specimen was graded on a scale of 0–3 during the SEM examination. A value of 3 was ascribed to typical control eyes and 0 when no trace of mucus was found. Two individuals did the assessments; the number of points then were totaled, and a mean was obtained of the “mucus index” for each treatment group.

Results

No visible signs of intolerance to AC were observed, except with the 20% AC solution; the latter induced marked ocular irritation after instillation. Macroscopic examination of the ocular surfaces showed a redness of the conjunctiva in treated eyes from this group. All other changes described hereafter were observed by SEM.

Conjunctival Surface

Under scanning illumination, the palpebral conjunctiva of untreated eyes had a wavy surface formed by the juxtaposition of irregular polygonal cells with marginal folds delineating the intercellular contacts (Fig. 1). At high magnification, the luminal membranes of epithelial cells were covered uniformly with dense microvilli. Goblet cell apertures, some of them extruding mucus droplets, were interspersed randomly between epithelial cells (Fig. 2). Mucus secretions present on the conjunctival surfaces were represented by disseminated droplets and a thin homogeneous mucus layer entrapping the microvilli of epithelial cells (Fig. 3).
Local administration of one 50-μl drop of 0.1 M AC (Group A) did not change either the appearance of epithelium or mucus secretions at 30 min postinstillation. After repeated application of this concentration every 5 min for 1 hr (Group B), the mucus layer covering the microvilli disappeared from extensive areas, resulting in a clear conjunctival surface (Fig. 4). However, isolated mucus secretions represented by droplets and strands were still present in some areas. In this group, AC effects were limited to the mucus layer, and the morphology of epithelial cells was preserved. Repeated application of 0.1 M AC over 2 hr (Group C) had similar effects on the mucus as those of Group B. Conjunctival surfaces were clear of the mucus layer, and only a few mucus droplets were present. In Group C, all treated conjunctivas showed focal cellular alterations, consisting of degeneration and desquamation of epithelial cells, and the presence of inflammatory cells (Fig. 5). Instillation of 20% AC every 5 min over 15 min (Group D) also resulted in the disappearance of the mucus layer and focal degeneration and inflammation of the epithelium. Changes observed in this group affected all treated eyes and were more severe and extensive than in other groups.

For a qualitative appraisal of the effects of AC on the conjunctival mucus, histograms were constructed to quantify (1) the mucus layer covering the conjunctival surface (Fig. 6A) and (2) the secretion of mucus (Fig. 6B), represented by mucus droplets. This evaluation showed good reproducibility of the control values, which were always consistently between 2.8–3.0, with the exception of one group. Thus, the histogram (Fig. 6A) showed that, compared with the control eyes, a dose-related reduction of the mucus layer occurred in the AC-treated eyes. At the same time, mucus secretion was almost unchanged, and the index only decreased to 2 with 20% AC (Fig. 6B).

**Corneal Surface**

The surface of the cornea of untreated eyes observed by SEM consisted of characteristic flat polygonal cells arranged in a mosaic pattern, in which light, dark, and intermediate cells were distinguishable (Fig. 7). High magnification revealed numerous microvilli covering the cell surfaces (Fig. 8). Table 2 summarizes the incidence and visual signs of toxicity of the cornea after instillation of the various AC solu-
**ACETYLCYSTEINE EFFECTS ON CONJUNCTIVA AND CORNEA**

**Thermes et al.**

**MUCUS LAYER COVERING THE SURFACE**

Fig. 6. (A) The "mucus index" of control and treated eyes calculated for the mucus layer covering the conjunctival surface (see conjunctival mucus for details). Three animals were used in each group. (B) The "mucus index" of control and treated eyes calculated for the secretion of mucus granules (see conjunctival mucus for details). Three animals were used in each group.

**SECRETION OF MUCUS**

Fig. 7. The corneal surface observed in untreated eyes consists of the juxtaposition of flat polygonal cells in which light (LC), dark (DC), and intermediate cells (IC) are distinguishable, ×500.

**Discussion**

The morphology of ocular structures has been investigated widely using light and transmission electron microscopy. More recently, SEM\(^1\) was used to study the normal and pathologic ultrastructure of ocular surfaces to evaluate the local tolerance of ophthalmic preparations. This technique has several advantages. It has a larger magnification range and better depth of focus and can provide detailed three-dimensional views of morphologic structures. The SEM was therefore the instrument of choice for the study of the disposition of mucus on the ocular surfaces.

The heterogeneous mucus layer, entrapping microvilli and mucus droplets randomly extruded from the conjunctival surface, is seen clearly in our SEM photographs. This mucus layer\(^6\) is spread over the globe during blinking and stabilizes the precorneal tear film between successive blinks.\(^1,17\) Our results demonstrate the mucolytic effect of AC on this conjunctival mucus layer. A dose-related effect of AC on the mucus layer was found, as illustrated by the histogram (Fig. 6A). Nevertheless, despite the fact that the ocular surfaces are clean after repeated AC treatment, mucus droplets continue to be secreted to protect the eye (Fig. 6B) against AC.

**Fig. 8.** High magnification shows microvilli covering the cell surface of the corneal epithelium, ×7500.
Table 2. Incidence and visual signs of toxicity of the cornea after instillation of acetylcysteine solutions

<table>
<thead>
<tr>
<th>Modifications observed in corneal epithelium</th>
<th>Number of animals with modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, n = 3</td>
<td>B, n = 3</td>
</tr>
<tr>
<td>Loss of microvilli</td>
<td>0</td>
</tr>
<tr>
<td>Cell degeneration</td>
<td>0</td>
</tr>
<tr>
<td>Cell desquamation</td>
<td>0</td>
</tr>
<tr>
<td>C, n = 3</td>
<td>D, n = 3</td>
</tr>
<tr>
<td>Loss of microvilli</td>
<td>2</td>
</tr>
<tr>
<td>Cell degeneration</td>
<td>2</td>
</tr>
<tr>
<td>Cell desquamation</td>
<td>3</td>
</tr>
</tbody>
</table>

* Treatment group.
† Number of animals.

The mechanism of action of AC on mucus was described earlier. In this previous study, the presence of free sulphydryl groups were found necessary to reduce the viscosity of mucoprotein solutions, and increasing the concentration of AC decreased the viscosity of the mucus. A lower mucus viscosity therefore would lead to a more rapid removal from precorneal and preconjunctival areas. Our observations by SEM agree with these earlier findings and provide further evidence for the effect of increasing AC concentration on mucus removal.

All four AC applications we administered affected the mucus to a greater or lesser extent. It would appear that, because of the continuous formation of mucus, regular 5-min instillations of AC are necessary to maintain a clean surface. The 1-hr treatment with 0.1 M AC (Treatment B) was effective in removing mucus without any evident toxicity. However, toxic effects of AC on ocular structures were seen after 2 hr (Treatment C) or with three drops of 20% AC (Treatment D). The 1.23 M AC solution (Treatment D) was hyperosmolar, and the 0.1 M AC solution (Treatments A–C) was almost isotonic. The toxicity may be caused by the hyperosmolarity of the solution, but others found no severe damage with a 6% NaCl hyperosmolar solution.

This is the first time that SEM has been used to demonstrate the superficial necrosis and cellular infiltration in the conjunctiva and cornea of the rabbit after instillation of 20% AC to our knowledge. Previous reports on the toxicity of AC did not agree with our findings. No adverse effects were found in studies on the rate of reepithelialization of the rabbit cornea with 10% and 20% AC after a superficial epithelial ulcer. By contrast, others observed that an intracorneal injection of 20% AC, which is available commercially, rapidly caused “in vivo” disintegration of the corneal stroma. Our results support the latter findings of toxicity of AC at this concentration.

In conclusion, SEM was used to show a dose-related effect of AC on the maintenance of the mucus layer. A 0.1 M solution of AC was adequate to dissolve the mucus deposited on the conjunctival and corneal surfaces by blinking. It was necessary to administer several applications to ensure a clean ocular surface. A satisfactory treatment, producing little observable toxicity, was 50-μl drops at 5-min intervals for 1 hr. Longer periods, or treatment with 20% AC, produced marked toxic lesions in rabbits. Because this species is known to be extremely sensitive to irritation, the relevance of these results to humans must be established with clinical data.

Key words: acetylcysteine, conjunctiva, cornea, mucus, scanning electron microscopy
Acknowledgment

The authors thank Madame Marjorie Cremont for preparing the manuscript.

References