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<Original Article>

Effect of combined sodium hyaluronate and carboxymethyl cellulose on ocular surface in rat dry eye model

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Abstract: This study was conducted to evaluate three different mixed formulations of sodium hyaluronate (SH) and carboxymethyl cellulose (CMC) using a low-humidity air flow-induced rat dry eye model and determine the most suitable mixture. The total thickness of the cornea, corneal epithelial thickness, corneal stroma thickness, damaged corneal epithelium percentage region, thickness of the bulbar conjunctiva epithelium, number of goblet cells, goblet cell occupation percentage region, and damaged bulbar conjunctiva epithelium percentage region were measured by histomorphological evaluation. After 5 h exposure to drying airflow, the thickness of the cornea and conjunctiva was decreased with desquamation of the corneal and conjunctiva epithelium. However, these dry eye symptoms were markedly inhibited by treatment with the reference and test formulations. More favorable effects on decreased thickness were detected in response to the CMC than the SH. However, SH had a greater protective effect against corneal and conjunctiva epithelial damage. The application of a mixture of 0.1% SH and 0.2% CMC showed more favorable effects on the corneal and conjunctival damage and the stabilization of the ocular surface than SH or CMC alone.

Keywords: carboxymethyl cellulose, dry eye, rat, sodium hyaluronate

Introduction

Dry eye or keratoconjunctivitis sicca (KCS) is a multifactorial ocular disease and the associated tear film instability can cause potential damage to the ocular surface [6]. Various etiologies lead to the development of dry eye conditions and, therefore, different methods have been used to induce experimental dry eye conditions or abnormalities in tear dynamic such as evaporation [4], pharmacologic blockade of cholinergic muscarinic receptors [7, 12], surgical excision of the lacrimal glands [13, 19], or mechanical prevention of blinking [13]. However, because these techniques cannot exclude the complex influence of surgery, hormone, and pharmacological agents, the low-humidity air flow-induced dry eye animal model was chosen for the present study [20].

Sodium hyaluronate (SH) is a naturally occurring substance found in connective tissue matrices of vertebrae such as synovial fluid. It is a linear polymer composed of long chains of repeating disaccharide units. SH is synthesized from corneal epithelial cells and, therefore, is a major component of tears [16]. Topical applications of SH have been proven to both subjectively and objectively improve the symptoms and non-invasive break-up time in patients with dry eye syndrome or KCS [10, 17, 24].

Carboxymethyl cellulose (CMC) is an essential high-molecular polysaccharide that prolongs the residual time of artificial tears on the ocular surface. Owing to its viscous and mucoadhesive properties, the use of CMC has been effective in the treatment of qualitative dry eye conditions and ocular surface staining [7]. CMC-based artificial tears have also been widely used after laser *in situ* keratomileusis to accelerate postoperative ocular surface recovery and minimize dry eye symptoms [18].

Although the mechanism underlying the profound protective role of CMC and SH on dry eye conditions is unknown, it is hypothesized that a combination CMC and SH formulation may have a beneficial effect on the ocular surface in dry eye. Therefore, the aim of this study was to determine the most suitable and efficacious formulation among three types of combination CMC and SH formulations, by using a rat dry eye model.

Materials and Methods

Animals and husbandry

Male Sprague-Dawley rats (6-week-old on receipt; SLC,

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Japan) were used after acclimatization for 7 days. The animals were housed at five rats per polycarbonate cage in a temperature (20–25°C) and humidity (40–45%) controlled room with a 12-h light:dark cycle. The animal feed (Samyang, Korea) and water were supplied *ad libitum*. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Kyungpook National University (2016-0016). After acclimatization, 10 rats per group (total 70 rats) were selected based on body weights and eyeball states (all animals with any defects in the eyeballs were excluded).

Experimental groups

The animals were divided into seven groups, which were treated as follows: groups 1 and 2, distilled water applied to the eye of normal and dry eye-induced rats (intact and dry eye [DE] control, respectively); groups 3 to 7, 0.5% CMC (Dial Clear; DHP Korea, Korea), 0.1% SH (Tearin Fresh; DHP Korea), 0.1% SH and 0.2% CMC mixture, 0.1% SH and 0.3% CMC mixture, and 0.1% SH and 0.5% CMC mixture applied to dry eye-induced rats (CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups, respectively).

Preparations and administration of test articles

Three different formulations, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC mixtures were used in this study. Samples (5-μL) of the test materials were instilled onto the rat eyeballs 30 min before exposure to airflow, and then every 30 min after initiation of exposure (total of 11 times, Table 1).

Induction of dry eye

The rats were anesthetized by the administration of 25 mg/kg intraperitoneal injections of zolazepam/tiletamine (Zoletile 50; Virbac, France). After deep anesthesia had been achieved, the central region of the corneal epithelium (0.4 mm^2) was scraped mechanically with an ophthalmic surgical blade. Then, the rats were placed in a desiccation room at a temperature of 28°C, relative humidity of 25 to 30%, and constant air flow (2.4 m/sec), and maintained for 5 h. The intact control rats were continuously exposed to the same conditions for acclimatization after the scraping procedure.

Histology and histomorphometry

The rats were killed with an overdose of zolazepam/tiletamine and the eyeballs with the bulbar conjunctiva were removed and fixed in Davidson's solution (37.5% ethanol, 12.5% acetic acid, and 25% formaldehyde [37% solution]). The corneal specimens were embedded in paraffin, cross-sectioned, and stained with hematoxylin and eosin (H&E) to examine the cornea and with periodic acid-Shiff (PAS) to detect mucus-producing goblet cells in the conjunctiva.

The total thickness of the cornea, corneal epithelial thickness, corneal stromal thickness, damaged corneal epithelium percentage region (%/5 mm of corneal epithelial lining), thickness of the bulbar conjunctiva epithelium at 1 mm from the limbus, number of goblet cells (numbers PAS-positive cells/μm of epithelial lining), goblet cell occupation percentage region (%/mm of epithelial lining), and damaged bulbar conjunctiva epithelium percentage region (%/mm of epithelial lining) were measured using a digital image analyzer (DMI-300; DMI, Korea). The histopathologist who performed the analysis was blinded to the group distributions.

Statistical analyses

Multiple comparison tests for different dose groups were conducted. The variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from the variance homogeneity, the data were analyzed using a one-way analysis of variance (ANOVA) followed by a least-significant difference multicomparison test to determine the pairs of group comparison that were signifi-

Table 1. Histomorphometrical changes detected in rat cornea after 5 h exposure to dry-air flow with or without test formulation instillation (5 μL/eye at 30-min intervals)

Groups	Thickness (μm)			Epithelial damage regions (% of 5-mm of cornea)
	Total	Epithelium	Stroma	
Controls				
Intact	355.164 ± 34.956	76.301 ± 7.624	277.628 ± 29.843	6.326 ± 4.113
Dry eye	268.501 ± 20.018[a]	53.335 ± 7.982[a]	237.409 ± 22.209[a]	30.505 ± 11.692[a]
Single formulas				
0.5% CMC	310.419 ± 43.824[ac]	64.147 ± 8.889[ac]	241.675 ± 32.466[b]	25.000 ± 7.468[a]
0.1% SH	295.914 ± 25.671[a]	56.282 ± 6.194[a]	253.626 ± 36.117	16.875 ± 6.256[ac]
Combination formulas				
0.1% SH + 0.2% CMC	323.418 ± 30.349[bc]	71.297 ± 13.719[c]	256.282 ± 29.995	12.640 ± 2.997[ac]
0.1% SH + 0.3% CMC	307.592 ± 30.961[ac]	73.097 ± 7.593[c]	254.456 ± 27.549	11.422 ± 3.996[bc]
0.1% SH + 0.5% CMC	288.356 ± 34.869[a]	61.491 ± 9.095[ad]	237.988 ± 46.541[a]	10.788 ± 5.035[c]

Values are mean ± SD of 10 rats. CMC, carboxymethyl cellulose sodium; SH, sodium hyaluronate; [a]$p < 0.01$ and [b]$p < 0.05$ compared with intact control. [c]$p < 0.01$ and [d]$p < 0.05$ compared with dry eye control.

cantly different. In the case of significant deviations from the variance homogeneity in Levene test, a non-parametric comparison test, Kruskal-Wallis H test was conducted. When a significant difference was observed in the Kruskal-Wallis H test, the Mann-Whitney *U* test was conducted to determine the specific pairs of group comparison that were significantly different. The statistical analyses were conducted using the statistical package for the social sciences (SPSS) for Windows release 6.1.3. (SPSS, USA). In addition, the percentage changes compared to that of the DE control were calculated to facilitate the understanding of the efficacy of test materials and the percent changes between intact and DE control were also calculated to observe the induction status as follows:

Equation 1. Percentage changes compared with intact control (%) = [({Data of DE control − Data of intact control} / Data of intact control) × 100]

Equation 2. Percentage changes compared with DE control (%) = [({Data of eye dropped groups − Data of DE control}) / Data of DE control) × 100]

Results

Changes in cornea

A focal desquamation of the corneal epithelial lining was detected in the DE control with significant ($p < 0.01$) decreases in the thickness of the cornea, corneal epithelium, and stroma, and significant ($p < 0.01$) increases in the damaged epithelial regions compared with the intact control. However, these histopathological changes to the cornea were decreased compared with the DE control in all three different mixed formulas-0.1% SH + 0.2% CMC mixture, 0.1% SH + 0.3% CMC mixture, and 0.1% SH + 0.5% CMC mixture and two single formulas-CMC and SH treated groups, respectively. The exception was the stroma thicknesses, in which no meaningful changes were detected after treatment with all five formulas tested in the present study. Compared with the DE control, the thickness of the cornea increased in the following ascending order to, 0.1% SH + 0.2% CMC, CMC, 0.1% SH + 0.3% CMC, SH, and 0.1% SH + 0.5% CMC. The thicknesses of the corneal epithelium were increased in this ascending order, 0.1% SH + 0.3% CMC, 0.1% SH + 0.2% CMC, CMC, 0.1% SH + 0.5% CMC, SH; and the damage to the epithelial regions decreased in this ascending order, 0.1% SH + 0.5% CMC, 0.1% SH + 0.3% CMC, 0.1% SH + 0.2% CMC, SH, and CMC (Fig. 1; Table 1).

The thicknesses of cornea were changed by −24.40% in the DE control compared with the intact control, but they were changed by 15.61, 10.21, 20.45, 14.56, and 7.39% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups compared with the DE control, respectively.

The thickness of the corneal epithelium was changed by −30.10% in the DE control compared with the intact control, but was changed by 20.27, 5.53, 33.68, 37.05, and 15.29% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups compared with the DE control, respectively.

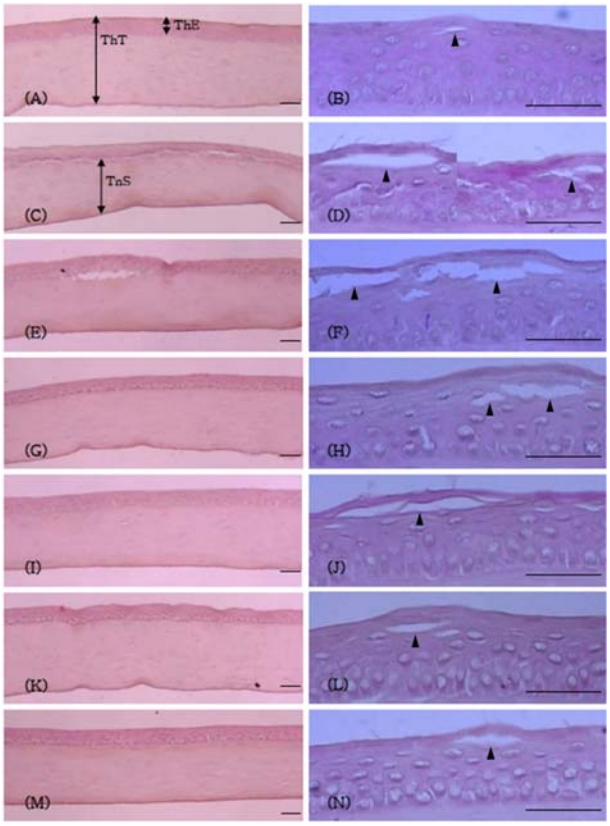

Fig. 1. Histopathological observations of cornea in intact control (A and B), dry eye control (C and D), CMC (E and F), SH (G and H), 0.1% SH + 0.2% CMC (I and J), 0.1% SH + 0.3% CMC (K and L), and 0.1% SH + 0.5% CMC (M and N) treatment groups. Arrows indicate thickness of total cornea (ThT), epithelium (ThE), and stroma (ThS). Arrowheads indicate region of cornea scraped mechanically. H&E stain. Scale bars = 80 μm.

The thicknesses of corneal stroma were changed by −14.49% in the DE control compared with the intact control, but was changed by 1.80, 6.83, 7.59, 7.18, and 0.24% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups compared with the DE control, respectively.

The damaged epithelial regions were changed by 382.19% in the DE control compared with the intact control, but were changed by −18.05, −44.68, −58.56, −62.56, and −64.64% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups compared with the DE control, respectively.

Changes on the conjunctiva

Compared with the intact control, focal desquamation of the bulbar conjunctiva epithelial lining was detected in the DE control with significant ($p < 0.01$) decreases in the conjunctiva epithelial thicknesses, number and percent of mucus-producing cells, and significant ($p < 0.01$) increases of the

damaged epithelial regions. However, these histopathological changes to the conjunctiva were decreased compared with those to the DE control treated with all five formulas in the present study, respectively. Compared with the DE control, the conjunctiva thickness was increased in ascending order in the 0.1% SH + 0.3% CMC, 0.1% SH + 0.5% CMC, 0.1% SH + 0.2% CMC, SH, and CMC; mucus-producing cell numbers were increased by 0.1% SH + 0.3% CMC, 0.1% SH + 0.5% CMC, 0.1% SH + 0.2% CMC, SH, and CMC; mucus-producing cell percentage was increased by 0.1% SH + 0.5% CMC, 0.1% SH + 0.3% CMC, 0.1% SH + 0.2% CMC, SH, and CMC; and damaged epithelial regions were decreased by 0.1% SH + 0.5% CMC, 0.1% SH + 0.3% CMC, 0.1% SH + 0.2% CMC, SH, and CMC (Fig. 2; Table 2).

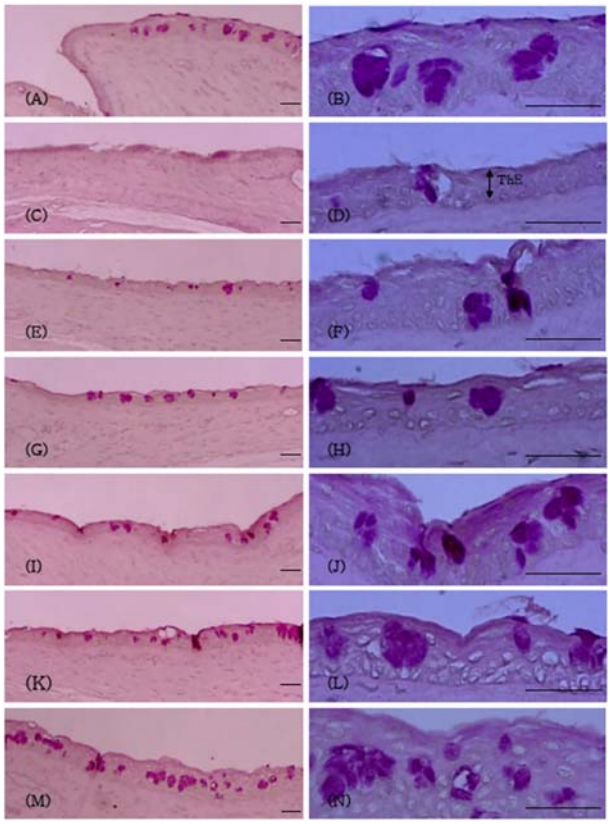

Fig. 2. Histopathological observations of conjunctiva in intact control (A and B), dry eye control (C and D), CMC (E and F), SH (G and H), 0.1% SH + 0.2% CMC (I and J), 0.1% SH + 0.3% CMC (K and L), and 0.1% SH + 0.5% CMC (M and N) treatment groups. Arrows indicate thickness of epithelium (ThE). Periodic acid-Shiff stain. Scale bars = 80 μm.

The thicknesses of conjunctiva epithelium were changed by −43.51% in the DE control compared with the intact control, but were changed by 20.39, 38.34, 51.19, 71.75, and 61.89% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups as compared with the DE control, respectively.

The numbers of mucus-producing cell were changed by −78.63% in the DE control compared with the intact control, but were changed by 108.00, 108.00, 184.00, 244.00, and 228.00% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups compared with the DE control, respectively.

The regions of the mucus-producing cells were changed by −80.37% in the DE control compared with the intact control, but were changed by 134.13, 174.57, 218.89, 225.41, and 227.37% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% groups compared with DE control, respectively.

The regions of damaged epithelium were changed by 822.24% in the DE control compared with the intact control, but were changed by −22.29, −55.73, −61.35, −67.29, and −71.24% in the CMC, SH, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC, and 0.1% SH + 0.5% CMC groups compared with the DE control, respectively.

Table 2. Histomorphometrical changes detected in rat conjunctiva after 5 h exposure to dry-air flow with or without test formulation instillation (5 μL/eye at 30 min-intervals)

Groups	Epithelial thicknesses (μm)	Mucus-producing cells		Epithelial damage regions (% among 1 mm of epithelium)
		Number (n/1 mm epithelium)	Regions (% of 1 mm epithelium)	
Controls				
Intact	58.431 ± 11.053	11.700 ± 3.529	28.647 ± 4.404	6.197 ± 3.545
Dry eye	33.006 ± 5.834[a]	2.500 ± 1.354[a]	5.623 ± 3.160[a]	57.151 ± 13.909[a]
Single formulas				
0.5% CMC	39.734 ± 6.231[ad]	5.200 ± 1.687[ac]	13.165 ± 3.385[ac]	44.413 ± 17.963[a]
0.1% SH	45.659 ± 5.254[ac]	5.200 ± 2.150[ac]	15.439 ± 4.568[ac]	25.299 ± 7.418[ac]
Mixed formulas				
0.1% SH + 0.2% CMC	49.902 ± 7.275[bc]	7.100 ± 2.132[ac]	17.931 ± 6.510[ac]	22.089 ± 6.618[ac]
0.1% SH + 0.3% CMC	56.685 ± 6.350[c]	8.600 ± 2.716[c]	18.298 ± 5.208[ac]	18.694 ± 6.869[ac]
0.1% SH + 0.5% CMC	53.433 ± 7.805[c]	8.200 ± 3.327[bc]	18.408 ± 9.652[bc]	16.437 ± 8.027[ac]

Values are mean ± SD of 10 rats. [a]$p < 0.01$ and [b]$p < 0.05$ compared with intact control. [c]$p < 0.01$ and [d]$p < 0.05$ compared with dry eye control.

Discussion

Both SH and CMC have been used to alleviate the symptoms of dry eye and prevent intraperitoneal adhesion after abdominal or gynecologic surgery [10, 14, 17, 21, 23, 25]. The mixed formulations of SH and CMC also exhibited an anti-adhesive effect and prevented the intra-abdominal adhesion formation [3, 5, 8, 9]. However, the effect of the mixed formulation of SH and CMC on dry eye has not yet been reported. Therefore, to determine the suitable formulation of the three different combination formulations of CMC and SH, their protective effects were evaluated in a low-humidity air flow-induced rat dry eye model in the present study.

The common histopathological feature of keratoconjunctivitis sicca is corneoconjunctival epithelial damage, which is desquamation of the surface epithelial linings with apoptosis [15, 20]. By 5-h exposure to dry airflow, desquamation of corneal and conjunctival epithelium was induced in the present study. The thickness of the cornea and conjunctiva markedly decreased, and the number of mucus-producing goblet cells was also reduced in a proportion of the regions with damaged corneal and bulbar conjunctiva epithelium when the dry eye was induced. However, these dry eye symptoms were inhibited by treatment with all three combination formulations, 0.1% SH + 0.2% CMC, 0.1% SH + 0.3% CMC and 0.1% SH + 0.5% CMC, and the two single formulas, CMC and SH.

These results were consistent with the findings of previous studies, showing that CMC had a beneficial effect on aqueous tear-deficient dry eye symptoms and ocular surface staining [11, 14]. In addition, our results agreed with previous reports that CMC reduced the incidence of epithelial defects during laser-assited *in situ* keratomileusis [1, 2], and that SH protected the corneal epithelium and reduced the histological changes in the corneal and conjunctival surface in dry eye [22, 26].

In summary, the 0.1% SH + 0.2% CMC combination exhibited more favorable effects on the total thickness of the cornea and the conjunctival damages than the SH and CMC did alone. Although the 0.1% SH + 0.5% CMC combination also showed a greater inhibition of the epithelial damages, its effects on the thicknesses were relatively lower. This observation suggests that an appropriate combination formulation of CMC and SH would exhibit favorable synergic effects in treating dry eye conditions.

Therefore, we suggest that the combination formulation containing 0.1% SH + 0.2% CMC has a favorable effect on dry eye conditions. Future studies involving the application of this formulation on ocular surfaces may further clarify its effects in the animal dry eye model.

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