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Preparation and analysis of sodium carboxymethyl cellulose and its effect on xerophthalmia

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carboxymethyl cellulose (CMC) has been effectively synthesized with high yield and purity. With the prolongation of time after model establishment, the values of Schirmer **I** test (S **I** t) and breakup time of tear film gradually decreased. The α-MSH and CMC alone could ameliorate corneal tissue damage and corneal morphological abnormalities in the dry eye to some extent. However, compared with monotherapy, the treatment of α-MSH combined with CMC has a more significant positive effect on the improvement of tear secretion and the stability of tear film in dry eye model in the early stage after modeling. There are significant differences between the model control group and the normal control group at each time point after model establishment (all $P<0.01$) at 7th, 14th, 21th and 28th days after treatment. After 7, 14 and 21 days treatment, the S **I** t values in the α-MSH+CMC treatment group were 4.80±0.79, 4.10±0.52 and 4.30±0.86 mm, respectively.

Keywords: Sodium carboxymethylcellulose, α-Melanocyte-stimulating hormone, Xerophthalmia, Dry eye model

Dry eye is a disease of the abnormal tear film and ocular surface caused by multiple factors, which is mainly manifested as the increase of tear film osmotic pressure and ocular discomfort, vision fluctuation, tear film instability, and other symptoms caused by ocular surface inflammation[1]. Studies have shown that the prevalence of dry eye is 5% to 34%, and the incidence is higher in women and the elderly population[2], especially. Improper treatment may lead to ocular surface inflammation, corneal damage, decreased vision, and even blindness. There are several different therapeutic methods in the treatment of dry eye, which includes the topical application of artificial tears, immunosuppressive agents, and non-steroidal drugs. The main functions of those methods depend on the promotion of the secretion of tears and inhibition of the inflammatory response, which can thereby relieveseveral symptoms of dry eye[3-5].

Sodium carboxymethylcellulose (CMC), a multifunctional polymer compound obtained by chemically modifying natural cellulose, has been widely used in the fields of food, medicine, and textiles. Notably, CMC, as an artificial tear, is clinically mainly applied for the treatment of the dry eye. CMC has a large content of anionic and hydrophilic groups, which has a good adhesiveness and has the capability of diluting inflammatory factors, stabilizing the tear film, and wetting the ocular surface. At the same time, artificial tears contain a variety of electrolytes, which can maintain the metabolic balance of ocular surface tissues and relieve several symptoms of dry eye. α-Melanocyte-stimulating hormone (α-MSH) is derived from an opioid-melanocyte-stimulating prohormone. Previous studies have confirmed that α-MSH can reduce dry eye symptoms and inhibit the production of inflammatory factors[6]. However, it is not clear yet whether α-MSH can positively promote the therapeutic effect of CMC on dry eye. In the current study, the co-treatment of α-MSH and CMC was proposed to treat dry eye induced by scopolamine hydrobromide. Meanwhile, the clinical indicators and the improvement of and morphological changes of keratoconjunctiva and keratoconjunctiva after the co-treatment of α-MSH and CMC were assessed.

Materials and Methods

Materials

Scopolamine hydrobromide (Sigma, USA); α-MSH (Calbiochem, USA); tear secretion phenol red cotton thread (Tianjin Jingming New Technology Development Co., Ltd, China.); fluorescein sodium

injection (Alcon, USA); periodic acid Schiff (PAS) kit (Roche, USA). Slit-lamp microscope (Chongqing Kanghua Technology Co., Ltd.); BX51 optical microscope (Olympus, Japan); FTIR Spectrometer (Thermo, USA).

Experimental animals

Six weeks old Wistar rats, half male and half female, weighing 160 to 180 g, were obtained by Experimental animal center of PLA Academy of military medical sciences. All the animals were housed in the clean animal laboratory of Tianjin Medical University, with free access to water and food, the indoor temperature of (25±1)℃, the relative humidity of (40±5)%, and 12 h light/12 h dark periodic light.

Results and Discussion

Synthesis of CMC (IR spectrum of CMC)

Crushing cellulose as a raw materials to 20 microns with a plant fiber pulverizer to obtain cellulose powder; 20 g of tetrabutylammonium fluoroborate and 2 kg of the pulverized cellulose powder were successively poured into 25 L of isopropanol solution and stirred to obtain the premix; then the subsequent reaction was carried out under nitrogen atmosphere. The premix is heated to 25℃, and then 9.1 L of sodium hydroxide solution (mass concentration of 10%) is added dropwise, and the basification reaction mixture is obtained after 0.5 h of reaction. The alkalization reaction solution is heated to 50℃ and reacted with 2.4 kg sodium monochloroacetate for 2 h to obtain the etherification reaction solution. The etherified reaction solution is naturally cooled to room temperature, and then added to the methanol solution for soaking for 8 h. 70% acetic acid was used for neutralize the aged reaction solution, adjusting pH to 6~8, then filter it to obtain filter residue. Washing the filter residue with ethanol with a volume concentration of 70% for 5 times and sodium carboxymethyl cellulose was obtained by drying the washed filter residue in a drying oven at 60℃ in isolated air, with a molecular weight of 778,000 and a viscosity of 52,415 mPa s, with a substitution degree of 0.91, and chlorine content of 0.18 wt%. CMC were well prepared with high yield and purity, and its corresponding FTIR spectra and structure diagram were shown in Figs 1 & 2, respectively.

Dry eye modeling, Grouping, treatment and examinations

The dry eye model was established based on the method described in the literature[6,7], 0.5 mL scopolamine hydrobromide (6 mL, dissolved in 0.9% NaCl solution) was subcutaneously injected alternately into the hind limbs at 9: 00, 12: 00, 15: 00, and 18: 00 every day for 28 days

60 animals were randomly divided into normal control group, model control group, NaCI treatment group, CMC treatment group, and α-MSH treatment group and α-MSH + CMC treatment group, 10 animals in each group. Apart from the normal control group, the dry eye model was established in all the other groups. From the first day after subcutaneous

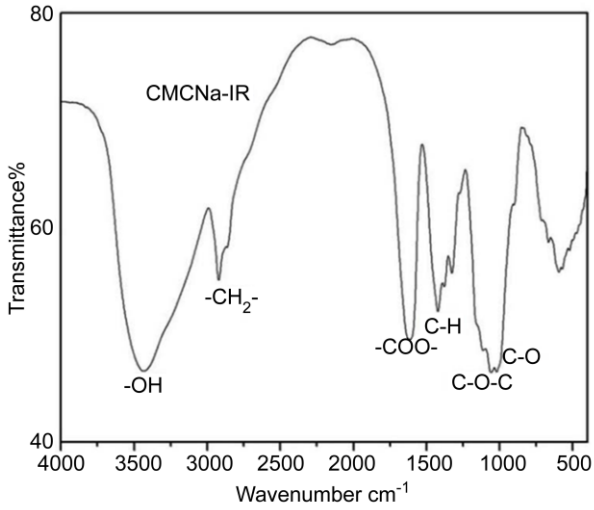

Fig. 1 — FTIR spectra of sodium carboxymethyl cellulose

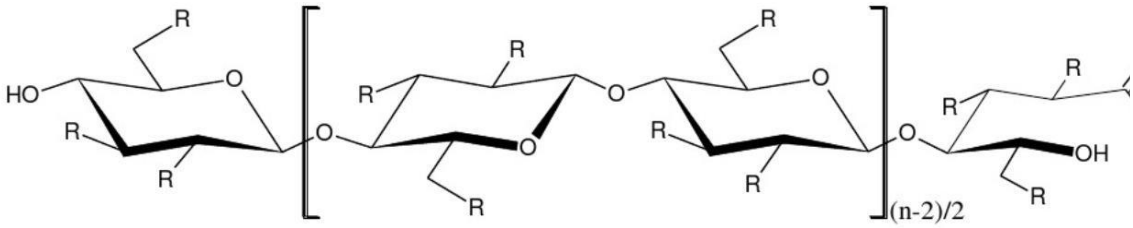

Fig. 2 — Structure diagram of sodium carboxymethylcellulose

injection of scopolamine hydrobromide on, normal saline, 0.5% CMC eye drops, normal saline containing 1 × 10^{-3}mg/mL α-MSH and 0.5% CMC eye drops containing 10 mg/mL α-MSH dropped into right eyes, respectively, according to the above groups with twice a day (8: 00 h, 17: 00 h), each time 25 μl, for 28 days, while the normal control group received no treatment. Each group underwent Schirmer **I** test**,** (S **I** t), breakup time of tear film (BUT), and corneal epithelial fluorescein staining before and on the 7th, 14th, 21th, and 28th days after the establishment of dry eye model. After the above examinations were completed on the 28th day after model establishment, 10% chloral hydrate (0.3 mL/100 g body weight) was injected intraperitoneally for general anesthesia. The eyeballs with upper eyelids, lower eyelids, and intact conjunctiva were taken and placed in 4% paraformaldehyde for fixation. Each group was examined at the same time point before and on the 7th, 14th, 21st, and 28th days after the establishment of the model.

The S **I** t value and BUT value of the right eye of the normal control group decreased slightly with time, and the S **I** t value of the model control group was slightly lower than that of the normal control group before modeling, but the difference was not statistically significant (P = 0.186). At 7th, 14th, 21th and 28th days after model establishment, the S **I** t value decreased gradually, and the S **I** t level at each time point was significantly lower than that of a normal control group, and the differences were statistically significant (all $P < 0.001$). The BUT values in the model control group were similar to those of the normal control group before modeling, and gradually decreased at 7th, 14th, 21th and 28th days after modeling, and the BUT at each time point was significantly lower than that of the normal control group, and the differences were statistically significant (all $P < 0.001$).

Changes in dry eye-related parameters

There were significant differences in tear secretion between the groups at different time points after intervention (F_{group} = 234.300, P = 0.000; F_{time} = 65.620, P = 0.000). The tear secretion o in the model control group gradually decreased with the prolongation of modeling time. Compared with the 7th day after treatment, except for the normal control group, the tear secretion in each group decreased, and the differences had statistical significance (all P < 0.001). There was no significant difference in the other time points before and after treatment in each group (all P > 0.05).At 7th, 14th, 21th and 28th days after treatment, the levels of S I t were significantly lower than those in normal controls (all P< 0.01).There was no significant difference in S I t values between the model control group and NaCl group at each time point after treatment (all P> 0.05).There was no significant difference in S I t value between the CMC group and the α-MSH + CMC group and NaCl group (all P > 0.05).At 7th, 14th and 21st days after treatment, the S I t value of α-MSH + CMC group was significantly higher than that of NaCl group (all P < 0.01), but there was no significant difference among NaCl group, CMC group and α-MSH group (all P> 0.05).28 days after treatment, there was no significant difference in S I t values between NaClgroup and CMC group, α-MSH group, and α-MSH + CMC group (all P> 0.05) as shown in Table 1.

Comparison of BUT values at different time points after treatment of each group

There were significant differences in the overall comparison of BUT values at different time points after intervention among the groups of rats (F_{group} = 540.000, P = 0.000; F_{time} = 30.030, P = 0.000). Compared with 7th d after treatment, apart from the normal control group, the BUT values of rats presented a reduction in each group, and the differences had statistical significance (all P< 0.01).

Table 1 — Comparison of S **I** t values at different time points after the administration of each group($\bar{x} \pm s$, mm)

Groups	Sample size	S **I** t values at different time points				
		0d	7d	14d	21d	28d
Normal control	10	7.250±1.244	7.156±1.247	5.031±1.958	5.047±1.073	5.100±0.835
Model control	10	6.111±1.711	4.050±0.826^{ac}	2.15O±O.490^{a}	2.100±0.681^{a}	1.975±0.638^{a}
NaCl	10	7.688±1.352	2.875±O.719^{ac}	2.375±O.619^{a}	2.532±0.957^{a}	3.321±1.067^{a}
CMC	10	7.300±1.160	4.200±0.422^{ac}	3.450±0.780^{a}	3.500±0.882^{a}	2.800±0.587^{a}
α-MSH	10	6.750±0.931	3.561±0.814^{ac}	3.562±0.479^{a}	3.50O±1.049^{a}	4.214±0.935^{a}
α-MSH+CMC	10	7.700±1.252	4.800±0.789^{abc}	4.100±0.516^{ab}	4.300±0.856^{a}	3.450±0.832^{a}

[Note: F_{group} = 234.300, P = 0.000; F_{time} = 65.620, P = 0.000. Compared with the normal control group at their respective time points, ^{a}P< 0.01; compared with the NaCl group of their respective time points, ^{b}P< 0.01; compared with the 0 d value within the group,^{c}P< 0.01 (two-way analysis of variance, Tukey test); S **I** t : Schirmer **I** test; CMC : sodium carboxymethyl cellulose; α-MSH : melanocyte stimulating hormone]

At 14th day after treatment, the BUT of the model control group, α-MSH treatment group, and α-MSH + CMC treatment group decreased compared with 7th day after treatment, and the differences had statistical significance (all $P< 0.01$). There was no significant difference between the groups before and after other time points (all $P> 0.05$).With the prolongation of time, the BUT values of the model control group gradually shortened, and the BUT values of the rats at each time point after modeling were significantly lower than those of the normal control group, and the differences were statistically significant (all P <0.01).There was no significant difference in BUT values between the model control group and the NaCl group at each time point (all P > 0.05).At 7th, 14th, 21th and 28th days after treatment, the BUT values of rats were significantly lower than that of the normal control group, and the differences were statistically significant (all $P< 0.01$). There was no significant difference in the BUT values between the CMC treatment group and NaCl treatment group (all $P> 0.05$).On the 7th day after treatment, the BUT of rats in the α-MSH treatment group as well as α-MSH + CMC treatment group was higher than that in the NaCl treatment group, and the differences were statistically significant (all $P< 0.01$).There was no significant difference in BUT values among the CMC treatment group as well as α-MSH treatment group and α-MSH + CMC treatment group at each time point (all $P> 0.05$) as shown in Table 2.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 statistical software (IBM, USA). The data of each test indicator in the current study were normally distributed by Shapiro–Wilk test expressed as $\bar{x} \pm s$. The variance of each group of data was equal by the Levene test. A completely randomized covariance study design was used. The differences of S **I** t value, BUT value and corneal fluorescein staining score between the normal control group and the model control group were compared using the independent sample t-test. The overall differences of S **I** t value, BUT value and corneal fluorescein staining score in the right eyes in the normal control group, model control group and various drug eye point groups at different time points after intervention were compared using two-way analysis of variance, and multiple comparisons were performed using Tukey test; $P< 0.05$ was considered statistically significant.

Results and Discussion

It is generally known that tear film components are composed of a watery liquid layer, a mucin layer, and a lipid layer. Meanwhile, the quality and quantity of each layer component and their intrinsic interactionall play an important role in maintaining the balance of the tear film[7].The pathogenesis of dry eye is extremely complex, and various pathogenic factors act on ocular surface tissues to cause an increase in tear osmolality, apoptosis of keratocytes, and atrophy of conjunctival goblet cells, which may have a negative impact on the secretion of the glands and nerve conduction[8-11].Dry eye is divided into tear evaporation overstrong type and underproduction type, thereby resulting in a large spectrum of induction methods of dry eye models, such as destroying animal meibomian glands, controlling environmental factors, applying epinephrine, atropine or glucocorticoids, removing lacrimal glands, destroying or interfering with nerve reflexes and so on, but the models elicited by these methods have several shortcomings including instability, excessive complications, etc. In the current study, scopolamine hydrobromide, a cholinergic M receptor blocker, was subcutaneously injected to simulate the dry eye model with reduced tear production, which has the characteristics of long-lasting effects and simple operation. Accordingly, this model elicited by scopolamine hydrobromide can be used to explore and study novel intervention measures and mechanisms of dry eye.In our research, we compared and investigated

Table 2 — Comparison of BUT values at different time points after treatment of each group ($\bar{x} \pm s$, s)

Groups	Sample size	BUT valuesat different time points				
		0 d	7 d	14 d	21 d	28 d
Normal control	10	8.406±1.188	6.125±1.454	4.813±1.203	3.844±0.884	3.467±0.681
Model control	10	8.750±1.020	3.800±1.056[ac]	2.000±0.324[ad]	1.700±0.470[a]	2.100±0.447[a]
NaCl	10	8.813±1.424	3.250±1.000[ac]	2.250±O.447[a]	2.125±0.342[a]	2.214±0.456[a]
CMC	10	8.300±1.767	4.000±0.816[ac]	2.400±0.699[a]	2.500±0.527[a]	2.300±0.483[a]
α-MSH	10	8.625±1.088	4.938±1.843[abc]	3.063±0.443[ad]	3.250±0.577[a]	3.143±0.663[a]
α-MSH+CMC	10	8.100±1.970	5.000±1.491[abc]	3.100±0.738[ad]	3.100±0.316[a]	2.900±0.316[a]

[Note: F_{group} = 540. 000, P = 0.000; F_{time} = 30. 030, P = 0.000. Compared with the normal control group at their respective time points, [a]$P< 0.01$; compared with the NaCl group of their respective time points, [b]$P< 0.01$; compared with the 0 d value within the group,[c]$P< 0.01$, compared with 7 d value within group, [d]$P< 0.01$ (two-way analysis of variance, Tukey test); BUT: breakup time of tear film; CMC: sodium carboxymethylcellulose; α-MSH: melanocyte stimulating hormone]

the therapeutic effect of α-MSH combined with CMC and other treatment in dry eye model elicited by scopolamine hydrobromide, and the combination of α-MSH and CMC in the early stage after modeling could significantly increase the tear secretion of dry eye model and alleviate the decrease of BUT in dry eye model than other groups. Intriguingly, CMC had no significant effect on tear film stability in the dry eye model, while the combination treatment of α-MSH and CMC presents a great alleviation of corneal injury in the dry eye model.

These studies have shown that the co-treatment of α-MSH and CMC can significantly enhance the relief of ocular surface function caused by dry eye, including increasing tear secretion, stabilizing tear film, and reducing corneal damage. However, it was found that the combination of α-MSH and CMC did not obviously enhance the alleviating effect of tear secretion on the 28th day in the dry eye model. After discreet discussion, we analyzed and speculated the possible reason resulting in this phenomenon was the progressive aggravation of damage to the ocular surface by intra peritoneal injection of scopolamine hydrobromide at a later stage, which exceeded the protective effect of the drug. In conclusion, the results of this experiment effectively indicate that the combination treatment of α-MSH and CMC exerts a synergistic and positive effect on the treatment of the dry eye.

At present, the treatment of dry eye is mainly based on artificial tears, and its key active ingredient is CMC[12]. CMC is an anionic cellulose water-soluble polymer with carboxyl groups, which has great adhesion. Besides, its anionic characteristics has a wide range of utilization, such as increasing the residence time on the ocular surface[13], promoting the binding to ocular surface cells, exert an essential role in promoting epithelial cell regeneration16, lubricating the ocular surface and stabilizing the tear film and so on[14,15]. Undoubtedly, drugs such as artificial tears and lubricants indeed have a positive effect on relieve the symptoms of dry eye, and sodium hyaluronate eye drops have been confirmed to have some efficacy in the treatment of dry eye in clinical studies, but they fail to cure or control dry eye etiologically. Studies have suggested that inflammation is a significant pathogenic factor in the development and progression of dry eye[16], while α-MSH has a powerful anti-inflammatory effect and can repair corneal epithelial injury effectively[17].In previous studies, Ru et al.[6] have proven that α-MSH could alleviate the reduction of tear secretion, increase tear film stability, inhibit apoptosis, reduce corneal edema, maintain corneal integrity, and inhibit the over expression of pro-inflammatory factors on the ocular surface by activating the PKA-CREB and MEK-ERK pathways. Moreover, it also can inhibit the expression of pro-inflammatory cytokines intercellular adhesion molecule-1 and tumor necrosis factor-α (TNF-α), and promote the expression of intercellular tight junction component occludin in the retina of streptozotocin-induced diabetic animals, which thereby reduce retinal vascular leakage and protect retinal function. The aforementioned results of our current study also showed that the combination of α-MSH with CMC could further improve the ocular surface signs of dry eye, which thus exertsan effect of cell protection and inhibition of corneal edema. Luger et al.[18] found that α-MSH inhibited proinflammatory factors in corneal injury, expression of corneal and uveal in an animal model of immune uveitis and the activation of inflammatory response pathways such as nuclear factor-KB. On the other hand, it also could regulate the proliferation, viability, and migration of inflammatory cells to some extent. In addition, clinical studies and animal experiments have confirmed that the expression of ocular surface inflammasomes and proinflammatory factors such as interleukin-1α and TNF-the α is up-regulated in the dry eye state[19-21], which may suggest that α-MSH has a great potential to enhance the therapeutic effect of CMC on dry eye by mean of inhibiting the expression and activation of inflammasomes.

Conclusions

In summary, the results of our study presented that the treatment of α-MSH combined with CMC could effectively alleviate the ocular signs of dry eye elicited by subcutaneous injection of scopolamine hydrobromide and exerted a more significant effect than α-MSH or CMC alone. Our study provides a new idea for further exploration and development of the intervention approaches of dry eye drugs, however, its specific mechanism of action remains to be further explored.

Conflict of interest

The authors declare no conflict of interests in this study.

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