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rhEGF/HP-β-CD complex in poloxamer gel for ophthalmic delivery

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Abstract

The purpose of the present study is to prepare chemically and physically stable rhEGF/poloxamer gel and to investigate its possibility of ophthalmic delivery. The rhEGF/HP-β-CD complex markedly increased rhEGF stability compared with rhEGF solution at 4 °C. The poloxamer gel was composed of poloxamer 407 (16%) and poloxamer 188 (14%). Additive of rhEGF/HP-β-CD complexes increased the gelation temperature and 0.5% rhEGF/HP-β-CD complex exhibited a suitable gelation temperature (35.5 °C). The gel strength and bioadhesive force decreased by increasing the rhEGF and HP-β-CD ratio from 1:4 to 1:20 in the complex. The in vitro release of rhEGF from poloxamer gel containing 1:4 rhEGF/HP-β-CD complex was much slower than that of rhEGF solution and faster than that of 1:20 rhEGF/HP-β-CD complex. After ocular administration of poloxamer gels in the rabbit, the concentration of rhEGF in tear declined at a first-order elimination. The poloxamer gel containing rhEGF/HP-β-CD complex increased the area under the concentration–time curve (AUC) of rhEGF in tear fluid compared with gel containing rhEGF solution. 1:20 ratio of rhEGF/HP-β-CD exhibited high AUC, indicating that rhEGF may be retained in the pre-corneal area for prolonged period. Therefore, the poloxamer gel could be applicable for the development of effective ophthalmic delivery.

Keywords: rhEGF; Hydroxypropyl-β-cyclodextrin; Complexation; Poloxamer; Ophthalmic delivery

1. Introduction

Human epithelial growth factor (rhEGF) is a single-chain polypeptide containing 53 amino acid residues (MW = 6045) and three disulfide bridges (Senderoff et al., 1994). rhEGF stimulates the proliferation and differentiation of epithelial tissues such as in the intestinal mucosa, corneal epithelial tissue, lung and trachea epithelial (Carpenter and Cohen, 1979). Moreover, rhEGF also inhibits gastric acid secretion (Bower et al., 1975; Elder et al., 1975; Gregory, 1975; Konturek et al., 1984).

Many attempts have been made to develop rhEGF preparations not only for the ophthalmic delivery system but also for oral delivery. However, the prevalent chemical reaction and physical instability of rhEGF have limited pharmaceutical development. rhEGF degrades via oxidation of

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the methionine residue (Rao et al., 1986), deamination of the asparagine residue (Ferraiolo and Benet, 1985) and succinimide formation of aspartic acid (Konturek et al., 1981). The most prevalent chemical reaction for rhEGF degradation is deamidation of the asparagine residue. The physical instability of rhEGF comes from the polymerization of monomer into dimer and trimer by disulfide exchange. The deamidation and aggregation of rhEGF could be prevented by nonionic surfactants such as Tween or polymers (Son and Kwon, 1995). Previously, we reported that the degradation of rhEGF was inhibited by the addition of bestatin, sodium caprate or sodium salicylate on the mucosal site (Han et al., 1998). Recently, cyclodextrin (CD) have been used to increase chemical and enzymatic stability of the peptides. CDs are groups of cyclic oligosaccharides which have been shown to improve physicochemical properties of many drugs through formation of inclusion complexes. Among the CDs, the β-CD and Hydroxy-β-cyclodextrin (HP-β-CD) exhibited the highest stabilizing effect for calcitonin and octreotide (Haeberlin et al., 1996). In particular, HP-β-CD is most commonly applied in aqueous eye drop formulations because of lower toxicity compared to parent CDs (Loftssona and Stefansson, 1997; Rajewski and Stella, 1996; Loftssona and Järvinen, 1999).

After ocular instillation, aqueous eye drop solution and suspension will mix with the tear fluid and be dispersed over the eye surface. The rapid loss of the instilled solution from the pre-corneal area will limit the ocular absorption. Therefore, prolonged corneal contact time of the applied drug is very important. The thermoreversible poloxamer gel system would be easy to administer with good patient compliance. It is also better retained in the eye than conventional eye drops avoiding rapid loss of the drug from the pre-corneal area. At concentrations above 20%, poloxamer solutions have unusual rheological characteristics (Schmolka, 1972), which permit them to be administered in liquid and to gel in situ, sol–gel transition temperature being lower than body temperature. The non-toxic properties and stability of poloxamers have been evaluated as an ophthalmic vehicle in animal models (Miller and Donovan, 1982; Edsman et al., 1998).

The purpose of the present study was to prepare a physically and chemically stable rhEGF poloxamer gel and to examine the possibility for an ophthalmic delivery system. Therefore, we prepared a rhEGF/HP-β-CD complex and investigated the stabilizing effect of HP-β-CD on rhEGF. Furthermore, we prepared a poloxamer gel containing a rhEGF/HP-β-CD complex and characterized the rheological properties. We also evaluated ocular availability of rhEGF after the administration of poloxamer gel into rabbit eyes.

2. Materials and methods

2.1. Materials

Highly purified recombinant rhEGF (more than 99% purity) was kindly provided by Daewoong Pharm. Co. (Seoul, South Korea). HP-β-CD was purchased from Sigma Chemical Co. (St. Louis, MO). Poloxamer 407 (P407) and Poloxamer 188 (P188) were purchased from BASF Co. (Ludwigshafen, Germany). All other reagents were of analytical grade.

2.2. Preparation and characterization of rhEGF/HP-β-CD inclusion complex

An inclusion complex was prepared by freeze-drying a solution of rhEGF and HP-β-CD in different molar ratios (molar ratio of 1:4, 1:10, 1:20). Briefly, rhEGF and HP-β-CD were dissolved in distilled water and filtered through a 0.22 μM filter. The filtrate was frozen and then freeze-dried at −70 °C for 24 h. A physical mixture was prepared by mixing freeze-dried rhEGF and HP-β-CD together using a mortar to obtain a homogeneous powder blend.

The rhEGF/HP-β-CD complex was characterized by thermal and spectroscopy methods. Thermal analysis was performed using a differential scanning calorimeter (DSC, Netzsoh, Model 200, Germany). Thermograms of the different samples (inclusion complex, physical mixture and pure substances) were obtained from a DSC equipped with a thermal analysis data system. Weighted samples (10 mg) were contained in holed alu-

minum pans and scanned at a rate of 10 °C/min, between −35 and 250 °C, using nitrogen as a purging gas.

KBr disks of the powdered samples were analyzed by a FT-IR spectrometer (Model-300E, Jasco Ltd, Tokyo, Japan). The data was obtained in the range of 400–4000 cm^{-1} for each sample.

2.3. Effect of HP-β-CD on the stability of rhEGF

The rhEGF and rhEGF/HP-β-CD complex were stored in tightly closed glass vials for 0, 1, 5, 7, 10, 20, 30, 60 days at 4 °C. Drug content was determined by HPLC.

2.4. Preparation of poloxamer gel

The poloxamer gels were prepared by the cold method as described by Schmolka (1972). P407 and P188 were dissolved in distilled water at room temperature and the solution was cooled down to 4 °C. The rhEGF/HP-β-CD complexes were then slowly added to the poloxamer solution with continuous agitation. The poloxamer gels were kept at 4 °C until use.

2.5. Measurement of gelation temperature

A 20 ml transparent vial containing a magnetic bar and 5 ml of poloxamer gel was placed in a water bath. A digital thermometer (Ika Labortechnik, RET digi-visc, German) connected to a thermistor was immersed in the poloxamer gel. The poloxamer gel was heated at a rate of 2 °C/min with constant (150 rpm) stirring. When the magnetic bar stopped moving due to gelation, the temperature displayed on the thermistor was determined as the gelation temperature (Choi et al., 1998; Miyazaki et al., 1991).

2.6. Measurement gel strength

Gel strength of the poloxamer gel was determined by the Simple Rheology Method (Shear test, Hansen and Gallo, 1990). Gel strength, is the viscosity of the poloxamer gel at physiological temperature. Measurements were carried out using a VT500 rotary viscosimeter (HAAKE) equipped with a coaxial cylinder HV1-DIN system thermostated at 37 °C. The shear rate was set at 93/s.

2.7. Determination of bioadhesive force

The bioadhesive force of poloxamer gel was determined by the peel test method (Instron® Method). In brief, a section of tissue was cut from the rabbit cornea and washed with saline. The corneal tissues were stored at 0 °C until use. Before experiments, the corneal tissues were immersed in phosphate buffer (pH 6.8) at 37 °C for 10 min. The corneal tissue was attached to an acrylic plate using a cyanoacrylate adhesive and the poloxamer gels were uniformly placed on the corneal tissue. Then, using an Instron® universal testing machine pressed the poloxamer gel with 5 mm/min cross-head speed and contacted for 10 min with 0.5 N pressure. The test machine was kept raised until the gel and cornea tissue was separated. Bioadhesive force, the detachment force was determined between the gel and the corneal tissue.

2.8. In vitro release studies

For the in vitro release studies, diffusion cells were used to evaluate rhEGF release from poloxamer gels (Cohen et al., 1997). The diffusion cells are comprised of two compartments separated by a non-limiting cellulose acetate membrane (0.8 μm pores, Milipore® S.A., 78054 Saint-Quetin, France), which does not constitute as a barrier against rhEGF diffusion. The donor compartment (Bottom) was filled with poloxamer gel with the rhEGF/HP-β-CD complex (2.5 ml), and the receiver compartment (Top) was filled with pH 6.8 phosphate buffer (3 ml). The temperature was maintained at 37 °C for all experiments. At the specified time, an aliquots (200 μl) of the medium in the receiver side was taken and replaced with an equal volume of fresh phosphate buffer. The collected samples were analyzed by HPLC.

2.9. In vivo ocular bioavailability

Male New Zealand albino rabbits weighing 2–3 kg were used in the in vivo experiments. A volume of 30 μl of poloxamer gel was dropped on to the cornea, and gently pulling away the lower eyelid allowed to the gel collect in the lower conjunctival sac of the rabbit's eye. In 20 s the lower eyelid was returned carefully to its normal position. Tear samples (2 μl) were collected without anesthesia using microcapillaries at appropriate intervals over a 12 h period (Bernatchez et al., 1993). The microcapillaries were then emptied, diluted and the concentration of rhEGF measured by the ELISA method.

2.10. rhEGF measurement

The HPLC system was composed of a model PU-980 pump, a model UV-975 UV–Vis detector, a model LC-Net II control borwin integrator and a model AS-950-10 autoinjector. Samples were injected into a 20 μl sample loop. Separation was achieved on a 10 μm reversed phase C_{18} column (Vydac; 2.5 × 250 mm^2, 10 μm). The mobile phase composed of acetonitrile, triethylamine and water (pH 6.5) in 250:2.2:850 (V/V/V) ratio. The flow rate was 1 ml/min and detection was monitored at 214 nm. The retention time of rhEGF was 10.8 min.

3. Results and discussion

3.1. Characterization of rhEGF/HP-β-CD complex

To confirm the formation of a rhEGF/HP-β-CD complex, each sample was analyzed by DSC and FT-IR spectrometry. The DSC results presented in Fig. 1 demonstrated an endothermic peak for rhEGF and HP-β-CD at 130 and 150 °C, respectively. The physical mixture thermograme was nearly identical to that of pure rhEGF and HP-β-CD. The inclusion complex shows a broad endothermic peak at 100 °C, but did not show pure endothermic peak of rhEGF and HP-β-CD. The disappearance of the endothermic peak of rhEGF or HP-β-CD and appearance of other endothermic peak, may indicate the occurrence of an inclusion complex between rhEGF and HP-β-CD.

Differences between the FT-IR spectra of the inclusion complex, physical mixtures and pure materials also indicate the interaction between rhEGF and HP-β-CD (Fig. 2). In particular, the characteristics of the carbonyl (C=O) absorption band at 1700 cm^{-1} for rhEGF moved to a lower wavelength in inclusion complexes. The physical mixture did not change. These results might be attributed to the dissociation of intermolecular hydrogen bonds in the rhEGF being replaced by weaker forces in the complexes.

3.2. Effect of HP-β-CD on the stability of rhEGF

Our previous study indicated that HP-β-CD exhibits stabilizing effect for rhEGF which containing 0.5% HP-β-CD (data not shown). Thus, prepared 1:4, 1:10 (0.5% HP-β-CD) and 1:20 complex and investigate the effect of HP-β-CD complex on the stability of rhEGF. rhEGF content determined by HPLC after storage for 6 months at 4 °C (Fig. 3). The 1:20 complex exhibited a very good stabilizing effect at 4 °C, indicating sufficient HP-β-CD inserted into peptide residue of rhEGF and protected rhEGF degradation. The stabilizing effect consists with Haeberlin et al.

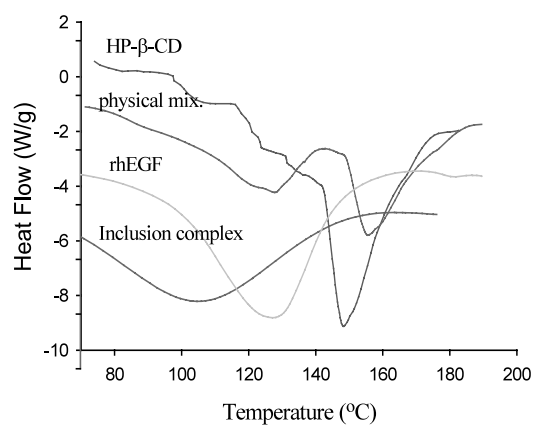

Fig. 1. DSC thermograms of freeze-dried rhEGF, HP-β-CD, physical mixture and rhEGF/HP-β-CD complex.

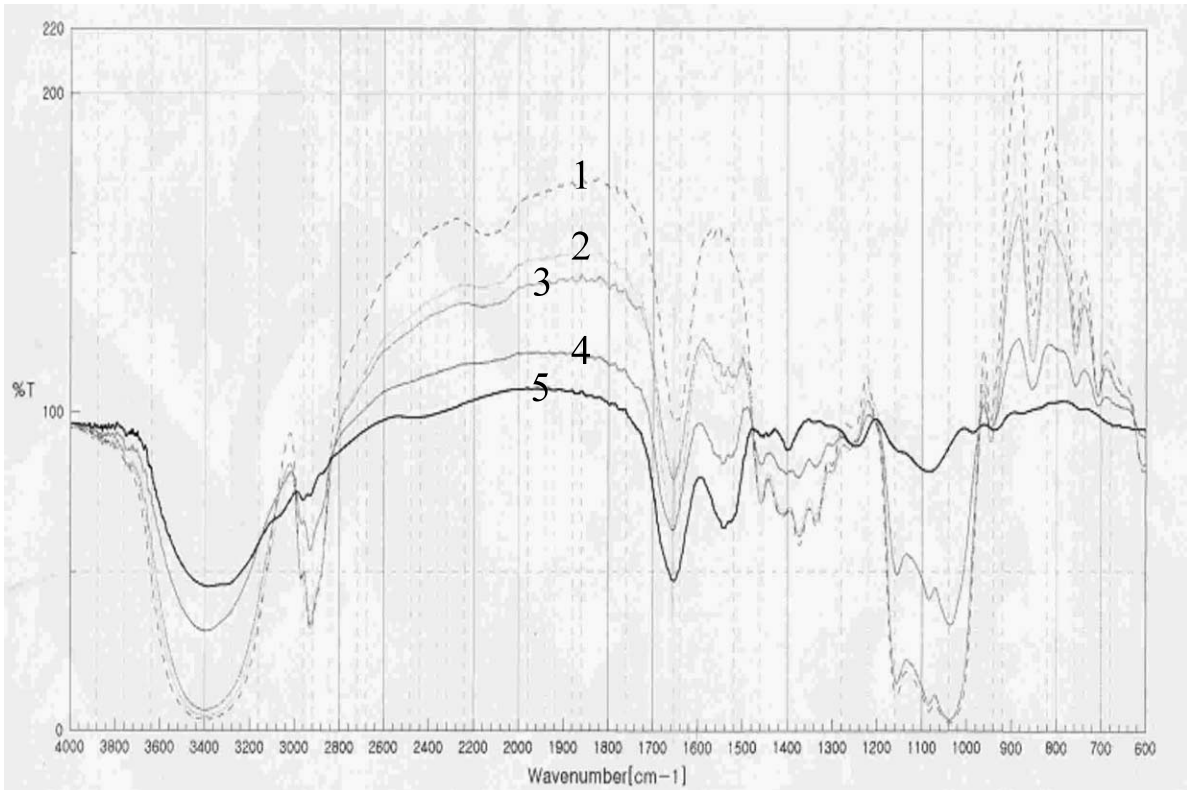

Fig. 2. FT-IR spectra of freeze-dried (1) HP-β-CD, (5) rhEGF and (2)–(4) rhEGF/HP-β-CD complex.

(1996) who reported the stabilizing effect of HP-β-CD on calcitonin and octreotide.

3.3. Effect of poloxamer composition on gelation temperature

Gelation temperature is the temperature at which the liquid phase makes a transition to a gel. A gelation temperature suitable for in situ gel would be 30–36 °C. An ideal in situ gel should be a free flowing liquid at room temperature to allow reproducible administration into the eye as a drop and also undergo in situ phase transition to form strong gel capable of withstanding shear forces in the cul-de-sac and sustain drug release at physiological condition.

Based on the gel with the suitable range of gelation temperature (30–36 °C), P407 and P188 were selected due to their thermo-sensitive gelling properties. In order to investigate the optimum concentration ratio of P407 and P188, various mixtures of poloxamer gel were prepared (Table 1). As the concentration of P407 was fixed at 12, 14, 16, and 20%, and the concentration of P188 varied from 10 to 30%, the gelation temperature decreased. When the concentration of P407 increased, the mixture needed smaller amounts of P188 to gel at the desirable gelation temperature. Among these compositions, one formulation of P407/P188 (16/14) was selected. The gelation temperature was 35.5 °C. The temperature dependent gelation of poloxamer solution could be explained by configuration change (Schick, 1966; Kramaric et al., 1992). Poloxamer molecules exhibit a well-arranged zigzag configuration. With increasing temperature, the zigzag configuration of poloxamer may be transformed into a close-packed meander configuration, forming a more close-packed and a more viscous gel.

The rhEGF/HP-β-CD complex also affected the gelation temperature of poloxamer gels. As shown in Fig. 4, with increasing concentrations of rhEGF/HP-β-CD from 0.1 to 1.0%, the gelation temperature slightly increased from 34.5 to 37.8 °C. At concentrations of 0.5%, the gelation temperature was 35.5 °C. This means that the formula is liquid at room temperature and turns into a gel instantly at physiological temperature. Thus, 0.5% rhEGF/HP-β-CD complex was used to prepare the poloxamer gel. Such a gelation temperature enhancing effect of rhEGF/HP-β-CD assumes that the peptide residue of rhEGF inserted into the poloxamer chains might disturb the micelles packing and entanglements of P407 and P188.

3.4. Physicochemical properties of poloxamer gel

The rhEGF/HP-β-CD complex prepared from various ratio of rhEGF and HP-β-CD were added to the poloxamer gel (P407/P188 = 16/14), and the physicochemical properties including gel strength and bioadhesive force were evaluated. The physical mixture was also examined. Gel strength was expressed with regard viscosity. As shown in Table 2, the viscosity and bioadhesive force did not changed at room temperature (23 °C), but temperature increased gelation temperature (37 °C), furthermore viscosity and bioadhesive force were increased for all formulations. rhEGF increased viscosity and slightly de-

Table 1
Gelation temperatures of poloxamer solutions

P407 Conc. (%, w/w)	P188 Conc. (%, w/w)	Gelation temperature (°C)
12	10	55.0
12	15	46.0
12	20	38.0
12	25	27.0
12	30	23.0
14	10	45.0
14	12	43.0
14	14	41.0
14	16	40.0
14	18	36.0
14	20	32.5
16	10	39.0
16	12	38.0
16	14	35.0
16	16	33.0
16	18	32.0
16	20	27.0
20	10	27.5
20	12	26.0
20	14	24.0
20	16	23.0
20	20	15.0

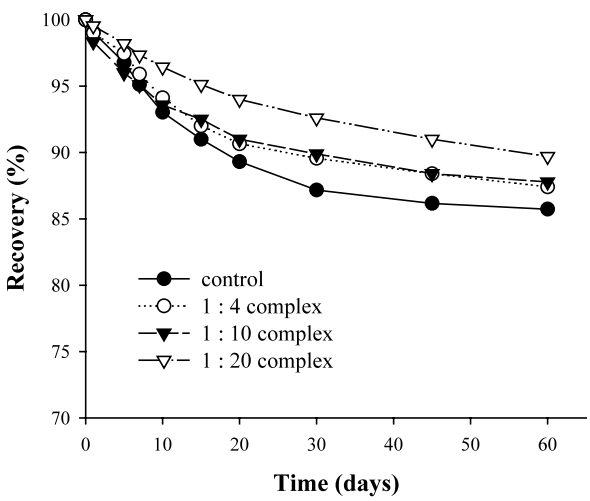

Fig. 3. Chemical stability of rhEGF in different formulations after 60 days at 4 °C. Each point represents the mean value ($n = 2$).

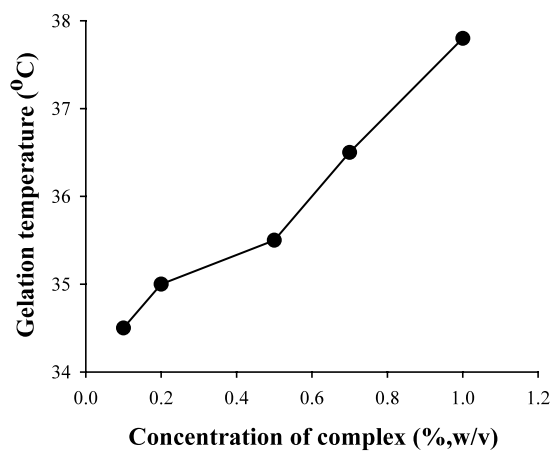

Fig. 4. Effect of rhEGF/HP-β-CD complex on the gelation temperature of poloxamer gels. The poloxamer gel is composed of P407/P188/complex (16/14/0.1–1.0%).

Table 2
Effect of rhEGF or rhEGF/HP-β-CD complex on the physicochemical properties of poloxamer gels

Formulations	Gel viscosity (mPas)		Bioadhesive force ($\times 10^{-3}$ N)	
	23 °C	37 °C	23 °C	37 °C
Control	207.9 ± 10.96	1946 ± 103.4	15.4 ± 2.5	51.2 ± 7.3
+rhEGF	159.9 ± 15.7	2367 ± 98.5	14.9 ± 4.7	47.3 ± 9.6
+Physical mixture[a]	172.6 ± 20.5	1550 ± 113.1	12.6 ± 4.3	30.6 ± 5.6
+1:4 complex[b]	186.6 ± 25.4	1775 ± 89.7	12.7 ± 5.4	34.2 ± 8.2
+1:10 complex[b]	186.6 ± 17.9	1567 ± 120.6	11.9 ± 3.7	27.4 ± 8.7
+1:20 complex[b]	175.9 ± 30.2	1189 ± 109.4	9.9 ± 5.7	15.4 ± 6.7

Each point represents the mean ± S.D. ($n = 3$).
[a] rhEGF and HP-β-CD ratio in physical mixture was 1:10.
[b] The different ratio express the rhEGF and HP-β-CD ratio in the complex.

creased bioadhesive force of the poloxamer gel. The viscosity-enhancing effect of rhEGF assumes that the peptide residue of rhEGF inserted into the poloxamer chains might disturb the micelles packing and entanglements of poloxamer. In addition, HP-β-CD complex decreased the viscosity and bioadhesive force and the 1:20 complex exhibited the lowest viscosity and bioadhesive force. As a possible mechanism by which HP-β-CD affected the physicochemical properties of gel, it is conceivable that the binding force (hydrogen bonding) of cross-linked reticular poloxamer gel became weaker by replacing HP-β-CD in the gel matrix. The phenomenon was similar to the decrease of gel strength and bioadhesive force of sodium salicylate (Yun et al., 1999).

3.5. In vitro release experiments

Fig. 5 illustrates the in vitro release of rhEGF from the poloxamer gel containing different ratios of the rhEGF/HP-β-CD complex and physical mixture at 37 °C. Overall release of poloxamer gel containing rhEGF solution was similar with poloxamer gel containing physical mixture and was much faster than that of the poloxamer gel containing rhEGF/HP-β-CD complex. As rhEGF and HP-β-CD ratio increased from 1:4 to 1:20 in poloxamer gel, the release of rhEGF tended to decrease. These results indicated that sufficient HP-β-CD inserted into peptide residue of rhEGF in 1:20 complex. Thus, rhEGF separated from the inclusion complex, and can be released from poloxamer gel. Therefore, rhEGF showed slower release in the 1:20 complex probably due to the HP-β-CD insertion.

3.6. In vivo ocular bioavailability

Fig. 6 illustrates the rhEGF concentration in the tear fluid as a function of time. The temporal profiles of tear concentration declined at a first-order elimination among the formulation studies.

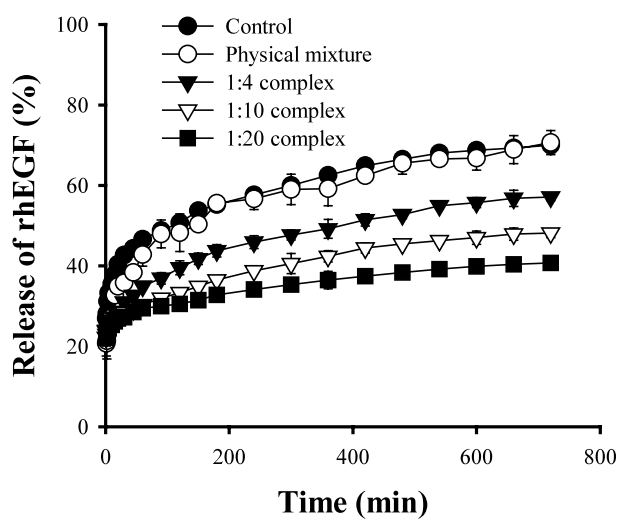

Fig. 5. Release of rhEGF from poloxamer gels. The poloxamer gel was composed of P407/P188 (16/14) and rhEGF (0.5%) or rhEGF/HP-β-CD complex (0.5%). Each point represents the mean ± S.E. ($n = 3$).

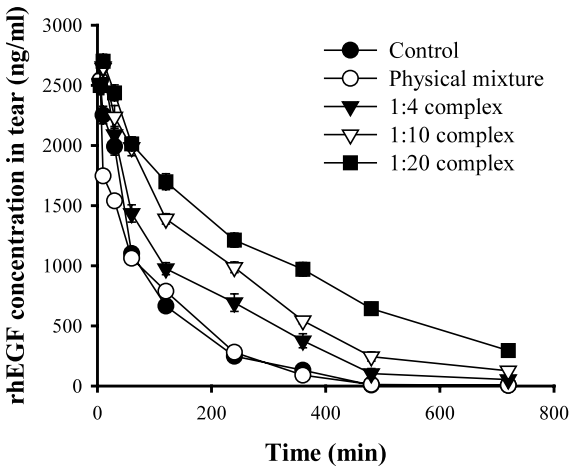

Fig. 6. rhEGF concentrations in tears after ocular administration of poloxamer gels. The poloxamer gel was composed of P407/P188 (16/14) and rhEGF (0.5%) or rhEGF/HP-β-CD complex (0.5%). Each point represents the mean ± S.E. ($n = 3$).

When poloxamer gel containing rhEGF solution and physical mixture, the AUC did not showed significant difference. The AUC of poloxamer gel containing the rhEGF/HP-β-CD complex was 1.6–3.8 folds greater than that containing rhEGF solution. When increasing the rhEGF and HP-β-CD ratio from 1:4 to 1:20 in the complexes, the AUC was increased from 362.36 to 852.79 μg min/ml, indicating that rhEGF may be retained in the pre-corneal area for prolonged period following instillation. However, the viscosity of the 1:20 complex was lower than that of the 1:4 complex (Table 2). Although, rhEGF and HP-β-CD affects gel viscosity and bioadhesive force, poloxamer gel has sufficient viscosity compared with rhEGF solution at the gelation temperature. Thus, the difference of the AUC is not due to the effect of gel viscosity. In vitro release studies (Fig. 5), indicated that sufficient HP-β-CD inserted into peptide residue of rhEGF and decreased release of rhEGF from complex. Therefore, sufficient HP-β-CD inserted into peptide residue of rhEGF in the 1:20 complex, sustained rhEGF release from the complex, due to prolonged resident time in the pre-corneal area.

Generally, prolonged drug residence time and improved drug penetration across the corneal barrier are important factors to increase ocular drug delivery. In the present study, the poloxamer gel containing rhEGF/HP-β-CD complex not only prolonged resident time at the pre-corneal area, but also maintained a high concentration of rhEGF in tears. However, increased pre-corneal retention of rhEGF in the pre-corneal area, dose not translated to an enhanced bioavailability in aqueous or the cornea.

4. Conclusion

In the present study, we prepared thermo-reversible poloxamer gel containing a rhEGF/HP-β-CD complex, which was easy to administer during ocular instillation and remained at the administered site. The rhEGF/HP-β-CD complex not only increased stability of rhEGF, but also sustained the release of rhEGF in the poloxamer gels. After instillation of the poloxamer gel into a rabbit eye, the gel prolonged the resident time at the pre-corneal area and maintained a high rhEGF level in the tears. Therefore, the poloxamer gel could be applicable for the development of effective ophthalmic delivery.

Acknowledgements

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References

Bernatchez, S.F., Tabatabay, C., Gurny, R., 1993. Sodium hyalutonate 0.25% used as a vehicle increases the bioavailability of topically administered gentamicin. Graefe's Arch. Clin. Exp. Ophthalmal. 231, 157–161.

Bower, J.M., Camble, R., Gregory, H., Gerring, E.L., Willshire, I.R., 1975. The inhibition of gastric acid secretion by epithelial growth factor. Experientia 31, 825–826.

Carpenter, G., Cohen, S., 1979. Epidermal growth factor (EGF). Annu. Rev. Biochem. 48, 193–216.

Choi, H.G., Jung, J.H., Ryu, J.M., Yoon, S.J., Oh, Y.K., Kim, C.K., 1998. Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int. J. Pharm. 165, 33–44.

Cohen, S., Lobel, E., Trevgada, A., Peled, Y., 1997. A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J. Contr. Release 44, 201–208.

Edsman, K., Carlfors, J., Petersson, R., 1998. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur. J. Pharm. Sci. 6, 105–112.

Elder, J.B., Ganguli, P.C., Gillespie, I.E., Gerring, E.L., Gregory, H., 1975. Effect of urogastrone on gastrin levels in normal subjects. Gut 16, 887–893.

Ferraiolo, B.L., Benet, L.Z., 1985. Peptides and proteins as drug. Pharm. Res. 2, 151–156.

Gregory, H., 1975. Isolation and structure of urogastrone and its relationship to EGF. Nature 257, 325–327.

Haeberlin, B., Gengenbacher, T., Meinzer, A., Fricker, G., 1996. Cyclodextrins-useful excipients for oral peptide administration? Int. J. Pharm. 137, 103–110.

Han, K., Choi, M.S., Chung, Y.B., 1998. Site-specific degradation and transport of recombinant human epidermal growth factor (rhEGF) in the rat gastrointestinal mucosa. Int. J. Pharm. 168, 189–197.

Hansen, E.E., Gallo, J.M., 1990. A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. Pharm. Res. 7, 491–495.

Konturek, S.J., Radecki, T., Brzozowski, T., 1981. Gastric cytoprotection by epidermal growth factor. Gestroenterology 81, 438–443.

Konturek, S.J., Cieszkowsk, M., Jaworek, J., Konturek, J., Brzozowski, T., Gregory, H., 1984. Effect of epidermal growth factor on gastrointestinal secretion. Am. J. Physiol. 246, G580–G586.

Kramaric, A., Resman, A., Kofler, B., Zmitek, J., 1992. Thermoreversible gel as a liquid pharmaceutical carrier for a gelenic formulation. Eur. Patent No. 0551626 (A1).

Loftssona, T., Järvinen, T., 1999. Cyclodextrins in ophthalmic drug delivery. Adv. Drug Del. Rev. 36, 59–79.

Loftssona, T., Stefansson, E., 1997. Effect of cyclodextrins on topical drug delivery to the eye. Drug Devel. Ind. Pharm. 23, 473–481.

Miller, S.C., Donovan, M.D., 1982. Effect of poloxamer 407 on the miotic activity of pilocarpine nitrate in rabbits. Int. J. Pharm. 12, 147–152.

Miyazaki, S., Nakamura, T., Takada, M., 1991. Thermo-sensitive sol–gel transition of Pluronic F-127. Yakuzaigaku 51, 36–43.

Rajewski, R.A., Stella, V.J., 1996. Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery. J. Pharm. Sci. 85, 1142–1169.

Rao, R.K., Thornburg, W., Korc, M., Matrisian, L.M., Magun, B.E., Koldovsky, O., 1986. Processing of epidermal growth factor by suckling and adult rat intestinal cells. Am. J. Physiol. 22, G850–G855.

Senderoff, R.I., Wootton, S.C., Boctor, A.M., Chen, T.M., Giordani, A.B., Julian, T.N., Radebaugh, G.W., 1994. Aqueous stability of human epidermal growth factor. Pharm. Res. 11, 1–48.

Schick, M.J., 1966. Configuration of the polyoxyethylene chain in bulk, nonionic surfactant. Surfactant Sci. 1, 753–793.

Schmolka, I.R., 1972. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. J. Biomed. Mater. Res. 6, 571–582.

Son, K., Kwon, C., 1995. Stabilization of human epidermal growth factor (rhEGF) in aqueous formulation. Pharm. Res. 12, 451–454.

Yun, M.O., Choi, H.G., Jung, J.H., Kim, C.K., 1999. Development of a thermo-reversible insulin liquid supposity with bioavailability enhancement. Int. J. Pharm. 189, 137–145.