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ORIGINAL RESEARCH

RETRACTED ARTICLE: A novel ocular delivery of brinzolamide based on gellan gum: in vitro and in vivo evaluation

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Background: The aim of the study was to develop a sustained ocular delivery of brinzolamide (BLZ) based on gellan gum.

Methods: The formulations were characterized for clarity, gelling capacity, rheological studies, pH, drug content, and in vitro drug-release behavior. In vivo rabbit eye irritation test was conducted to evaluate irritation of the BLZ gel drug-delivery system. The prepared BLZ formulations were then investigated in vivo and compared with commercially available BLZ eyedrops with regard to pharmacodynamics.

Results: The results showed that the optimum concentration of gellan gum was 0.25% w/v; the prepared liquid was converted into a flowing gel after the addition of simulated tear fluid. In vitro release profiles showed that the release of BLZ from the in situ gel exhibited sustained characteristics. Draize test results showed that BLZ in situ gels did not stimulate signs of eye tissue activity and were less irritating than BLZ solutions and commercial Azopt.

Conclusion: The results of pharmacodynamics implied that the novel preparation of BLZ in situ gel effectively prolonged intraocular pressure-lowering effect after administration.

Keywords: in situ gel, pH sensitive, glaucoma, ocular drug delivery, sustained release

Introduction

Glaucoma is the most common eye disease in the world and is a leading cause of irreversible blindness.[1] Because of increase in intraocular pressure (IOP), glaucoma can lead to progressive loss of vision in the optic disc, usually without symptoms. Glaucoma is reported to be the result of an imbalance between aqueous humor drainage and IOP reduction, and brinzolamide (BLZ) is one of the most effective treatments for glaucoma.[2]

BLZ is a non-competitive, effective, and very specific carbonic anhydrase inhibitor for the treatment of glaucoma.[3] Clinically, BLZ ocular suspension was mainly used as a first-line antiglaucoma medication following a dosage of one drop each time and twice a day (preferably three times a day). However, its use has been limited by a number of factors, including systemic adverse events (taste aversion), ocular adverse events (eye burning sensation and stinging pain), and relatively high prices.[4] Therefore, our group intended to develop a suitable eye preparation to improve the efficacy of BLZ.

Gellan gum (GG) is a high molecular weight bacterial extracellular polysaccharide secreted by *Pseudomonas elodea*. It is an anionic polysaccharide made of a tetrasaccharide repeating unit of one α-l-rhamnose, one β-d-glucuronic acid, and two β-d-glucoses as reported earlier.[5] GG was originally a food additive that acted as a stabilizer, thickener, and gelling agent in a wide variety of foods. More recently, GG has been investigated as a material for biomedical applications due to its biocompatibility and low cytotoxicity.[6,7]

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GG hydrogels are produced by physical cross-linking methods induced by temperature variation and the presence of positive ions. They exhibit a conformational transformation from the disordered state (single chain) to the ordered state (double helix) as temperature decreases. The gelation is considered to be mediated by the double-helix formation and the aggression of such helices into a three-dimensional structure. Metallic cations are needed to electrically shield the carboxyl groups and to allow a tighter aggregation of the helices.[8,9] Because of the phase transition properties, it has been used for oral,[10,11] ocular,[12–14] and nasal delivery systems.[15,16] The current effort is focused on the design and evaluation of systems that can be provided in the form of droplets without causing blurred vision or irritation. This will provide a suitable adhesion force to improve the retention time and slow release effect, and increase therapeutic efficacy and patient compliance.[17,18] Therefore, the in situ gel system has been developed as an ideal eye formula that, after the sol-gel phase transition, is subject to physiological conditions present in the eye. The original eye solutions, suspensions, and ointment formulations are clearly insufficient to combat these diseases, and efforts to design and develop better treatment systems are the main focus of this study.

In this study, for the first time, BLZ in situ gel was prepared by using a natural polysaccharide, GG. A pharmaceutical evaluation of BLZ gels was conducted subsequently with regard to clarity, gelling capacity, rheological studies, pH, drug content, and in vitro drug-release behavior. In vivo rabbit eye irritation test was carried out to evaluate irritation of the BLZ gels drug-delivery system. Finally, the pharmacodynamics of BLZ gels were also investigated.

Materials and methods

Materials

BLZ was provided by Bellen Biopharma Co., Ltd. (Wuhan, People's Republic of China; batch number: 20160312). BLZ eye drops (Azopt) (10 mg/mL) were purchased from Alcon Laboratories Inc. (Fort Worth, TX, USA). GG was obtained from Kelco Chemical Co., Ltd. (Shanghai, People's Republic of China) (500 kDa, 95% deacetylation). The purified water used was prepared using the by Milli-Q system (Millipore, Bedford, MA, USA). All other chemical reagents used in the study were of high-performance liquid chromatography (HPLC) or analytical grade.

Animals

In this study, all animals were purchased from the animal center of Shanghai Jiaotong University Medical School. All experiments were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the China National Institutes of Health (Shanghai, People's Republic of China). Legal approval to perform the study was obtained from Jiaotong University School of Medicine. All procedures performed in studies involving animal experiment were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration. New Zealand rabbits (2.0–2.5 kg) were placed in the standard animal cages at a constant temperature of 22°C±2°C and relative humidity of 50%±5%. They were fed with food and water according to standard practice.

Preparation of ocular in situ gel

Ion-sensitive situ gels were prepared using three different concentrations of polysaccharide. Aqueous GG solutions (0.25%, 0.5% and 1.0%) were prepared by dissolving a specified amount of GG in distilled water under magnetic stirring at 90°C. The concentrations of the polymer were selected based on a previous work (Table 1).[14] The drug solution (BLZ, 1%) was gradually added through a micropipette into the aqueous GG solution placed on a magnetic stirrer. Then, proper dispersant and preservative were slowly added to the system and mixed well for at least 30 minutes. The pH of the formulation was between 6 and 8.

Characterization of the prepared gels

Clarity

The clarity test was observed by visual inspection under good light and against a black and white background with the contents set in motion by a swirling action. Also, formation of turbidity or any unwanted particles dispersed in the solution were observed for.[19]

Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a beaker containing 50 mL of freshly prepared tear fluid (TF) solution and was visually observed for gelling time. TF (pH =7.0)

Table 1 The composition of in situ gel formulations

Formulation	BLZ (w/v) (%)	GG (w/v) (%)	Mannitol (w/v) (%)	Chlorhexidine acetate (w/v) (%)
Gel A	1	0.25	5	0.01
Gel B	1	0.5	5	0.01
Gel C	1	1.0	5	0.01

Abbreviations: BLZ, brinzolamide; GG, gellan gum.

Table 2 Coding for the gelling capacity

Observation	Coding
No gelation	–
Gelation occurred in few minutes (5 min) and remained for few hours (1 h)	+
Gelation immediate (30 s), remained for few hours (4 h)	++
Gelation immediate (30 s), and for extended period (permanent)	+++
Very stiff gel (gel formation immediately and permanently)	++++

consisting of NaCl 6.78 g, $CaCl_2 \cdot 2H_2O$ 0.084 g, KCl 1.38 g, and $NaHCO_3$ 2.18 g in 1,000 mL of purified water was prepared according to an earlier report.[20] Coding for the gelling capacity is described in Table 2.[21]

Rheological studies

The viscosity measurements were carried out using NDJ-5s viscometer. The developed formulations were placed in the sampler tube using spindle no. 2. Viscosity of the prepared formulations was measured using the research rotator and oscillatory rheometer. Then, the viscosity of the developed formulations in gel made with TF was determined by a rotational viscometer using a proper sample. The sample was placed in a small holder and the spindle was lowered perpendicularly into it. The spindle was rotated at varying speeds and a suitable speed was selected. Rheological studies of formulation are shown in Table 3.

Measurement of pH

For each formulated batch, the pH value was measured using a pH meter that was previously calibrated using standard buffers of pH =4.0 and pH =7.0 as per the established procedure.[22]

Drug content

A total of 1 mL of the developed formulation was dissolved in 100 mL phosphate buffer (pH =7.4) before using HPLC to determine drug concentration. The concentration of BLZ was determined by HPLC (Figure 1). Separation was carried out at 30°C using a reverse-phase C18 column (5 µm, 4.6×250 mm). The mobile phase consisted of methanol and water (60:40, v/v). The detection wavelength was 257 nm, and a flow rate of 1.0 mL/min was employed. A sample volume of 20 µL was injected. Appearance, pH, gelling capacity, and drug content (results of in situ gel) are shown in Table 3.

Stability studies

Selected ocular formulations (Gel B) were stored at 4.0°C±1.0°C, room temperature (25°C±1°C), and 40°C±1°C, respectively, for 3 months. At the end of the first, second, and third month, the clarity, pH, gelling capacity, and drug content of the formulations were evaluated.

In vitro release studies

Dialysis bag method was used for the in vitro study. The BLZ in situ gel at a volume of 1 mL (or BLZ eye drops 100 µL) was directly put into the dialysis bag (MWCO =10,000), and then 5 mL of TF was added. The release medium was 18±0.5 mL of fresh TF solution. In order to simulate the eye temperature, the vibration temperature was set at 35°C±0.5°C and the stirring speed was kept at 50 rpm. Samples (2 mL) were removed from the release medium at intervals of 0, 1, 2, 4, 6, 8, 10, and 12 h and replaced with an equal amount of fresh TF solution. The concentration of BLZ was determined by liquid chromatography. The cumulative release percentage was calculated, and a graph of percentage cumulative release against time was plotted.

In vivo rabbit irritation test

All the animals used were the of same batch number and had no signs of inflammation or visual abnormalities such as cataracts or glaucoma. A single-dose eye stimulation test was conducted in twelve healthy New Zealand white rabbits before they were divided into four groups. In vivo eye irritation tests of BLZ in situ gel were performed in five New Zealand rabbits. All tests were performed in the same laboratory with continued artificial lighting. After 60 minutes of acclimatization in restrainer boxes, 50 µL of the BLZ in situ gel formulation was instilled into the

Table 3 Rheological studies of formulation and characterization of the prepared gels

Formulation	Before gelation	After gelation				
	Viscosity of solution (mPa s)	Viscosity of in situ gel (mPa s)	Appearance	pH	Gelling capacity	% drug content
Gel A	200	480	Transparent solution	6.54	+	85.2
Gel B	500	2,200	Transparent solution	6.32	+++	84.8
Gel C	6,000	8,000	Transparent solution	6.06	++++	86.2

Notes: +Gelation occurred in few minutes (5 min) and remained for few hours (1 h); +++gelation immediate (30 s), and for extended period (permanent); ++++very stiff gel (gel formation immediately and permanently).

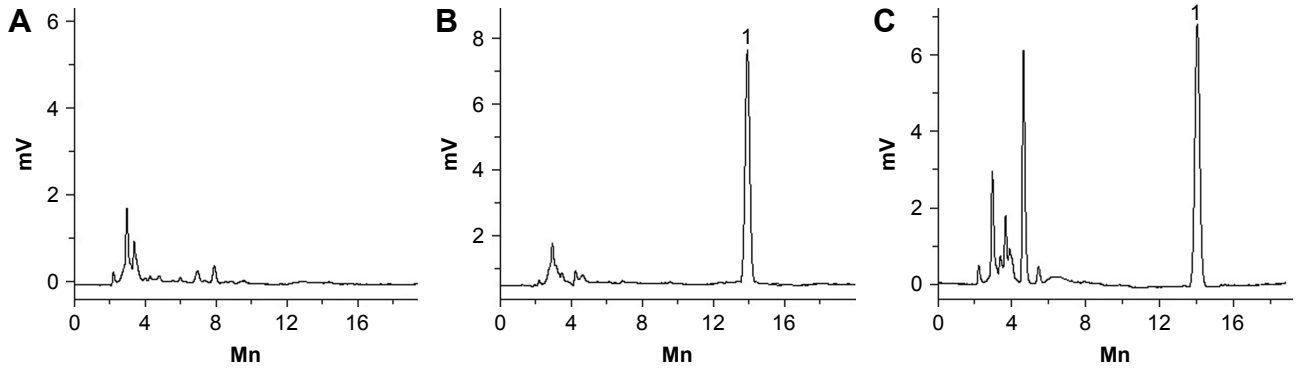

Figure 1 Chromatograms of BLZ in phosphate buffer solution.
Note: (**A**) Blank; (**B**) standard sample; (**C**) sample.
Abbreviation: BLZ, brinzolamide.

conjunctival sac of the rabbit's right eye; the left eye was not manipulated (control).

The tested eyes were observed at 1, 2, 4, 12, 24, 48, and 72 h to compare changes in cornea, iris, and conjunctiva secretion with control of bulbar conjunctival edema. The eye irritation levels were scored using the modified Draize test.[23] Long-term irritation tests were the same as those of single-dose eye irritation, but lasted 7 days. After irritation tests, the rabbits were killed by air injection. The eyeball was fixed in 10% formalin and stained with hematoxylin eosin. Histopathological changes of conjunctiva were observed under the microscope.

Pharmacodynamic studies

New Zealand White rabbits were used for in vivo pharmacodynamic studies. Before the experiment, the rabbits were placed in the dark room for 5 h. IOP was measured with a tonometer (YZ7A; Shanghai Huanxi Medical Technology Co Ltd, Shanghai, People's Republic of China) under surface anesthesia (0.2% lidocaine). The 12 rabbits were divided into three groups: one instilled with 50 µL BLZ in situ gel into the left eye, the second instilled with an equal amount of BLZ solution into the left eye, and the third instilled with 50 µL physiological saline into the left eye. In order to avoid experimental deviation, the right eye conjunctiva sac (control) was placed with 50 µL physiological saline and maintained for about 1 minute to prevent eye drops from overflowing. IOP was measured eight times at the scheduled time intervals (0, 0.5, 1, 2, 3, 4, 5, and 6 h). Each measurement was repeated three readings.

Results and discussion

Characterization of the prepared gels

In order to better understand the characterization of the gel, gel formation in vitro was observed. The two main prerequisites for an in situ gel are optimum viscosity and gelling ability. The formula should have an optimum viscosity to make the liquid easy to fall dropwise and then rapidly undergo a sol-gel transition due to ion interactions. Figure 2 shows the gel forming under the conditions of TF. The aim of the present investigation was to formulate an in situ gel. We already know that gels show thixotropic behavior; so, rheological studies should be performed.

The formulations (Gels A, B, and C) were prepared using various concentrations of GG. All the formulations prepared were clear without any turbidity, suspended particles, or impurities. The pH value of the in situ gel solution was found to be between 6.06 and 6.54 for all the formulations.

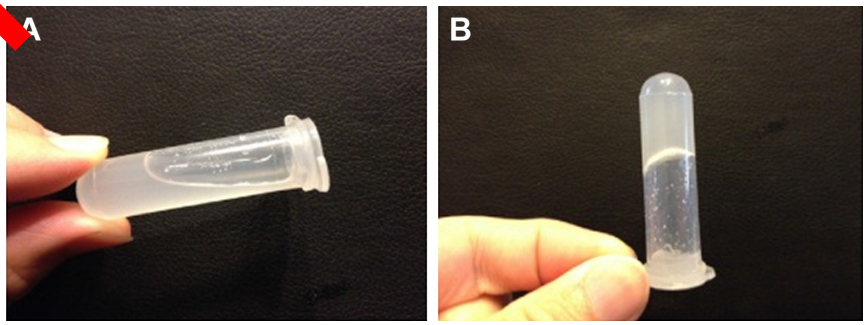

Figure 2 In vitro hydrogel formation with in situ gels (0.5% GG, w/v) (**A**) and TF (**B**).
Abbreviations: GG, gellan gum; TF, tear fluid.

The formulation Gel B had a pH value of 6.32, which was acceptable for ocular preparations. Gelling capacity is coded as shown in Table 2. Gel B shows immediate gelation for an extended period.

As shown in Table 3, the viscosity of the gel increased with increasing GG concentration. At low concentrations (0.25% and 0.5%), a greater viscosity change was found when GG underwent sol-gel transition. In contrast, as 1.0% GG obtained relatively high initial viscous solutions, the viscosity changes observed after gel formation were limited. The developed formulation Gel B yielded good results and was therefore selected as the optimized batch for the following studies. The viscosity of the test gel increased with higher GG concentrations. It was proposed that as the concentration of GG increased, the polymer chains approached closer, and the number of interactions between the polymer chains increased, leading to a denser 3-D network structure. When the concentration of GG reached 1.0%, high viscosity made administration with a conventional nebulizer difficult.

Stability studies

Table 4 shows the results of the stability study on in situ gel BLZ (Gel B) in the third month. No obvious change in pH value (about 6.3) or gelling capacity during the 3 months was observed.

Table 4 The stability studies of the BLZ in situ gel in the observation period

Months	Temperature	Appearance	pH	Gelling capacity	% drug content
0	4°C±1°C	Transparent solution	6.30	+++	84.3
	25°C±1°C	Transparent solution	6.31	+++	84.8
	40°C±1°C	Transparent solution	6.29	+++	84.1
1	4°C±1°C	Transparent solution	6.32	+++	84.2
	25°C±1°C	Transparent solution	6.31	+++	84.5
	40°C±1°C	Transparent solution	6.30	+++	83.9
2	4°C±1°C	Transparent solution	6.29	+++	84.2
	25°C±1°C	Transparent solution	6.29	+++	84.4
	40°C±1°C	Transparent solution	6.31	+++	84.0
3	4°C±1°C	Transparent solution	6.31	+++	84.2
	25°C±1°C	Transparent solution	6.32	+++	84.3
	40°C±1°C	Transparent solution	6.28	+++	83.8

Note: +++Gelation immediate (30 s), and for extended period (permanent).
Abbreviation: BLZ, brinzolamide.

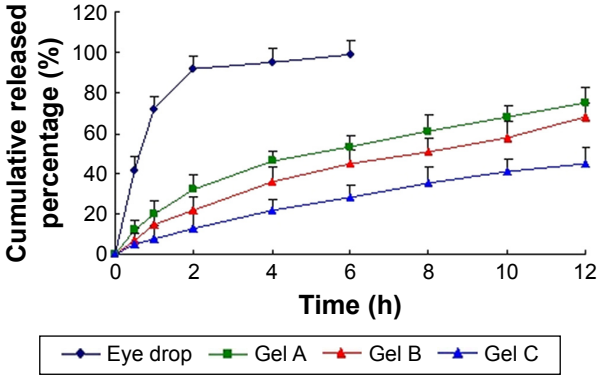

Figure 3 In vitro release profiles of BLZ in situ gels from three batches.
Notes: Release experiments were carried out in fresh TF solution as a dissolution medium at 35°C±0.5°C. Each point represents the mean value of three different mean ± SD.
Abbreviations: BLZ, brinzolamide; TF, tear fluid.

In vitro release studies

Figure 3 shows the cumulative amount of BLZ released versus time profiles for the BLZ marketed eye drop and in situ gels (Gels A, B, and C) with different concentrations. As shown in Figure 3, 42% BLZ drugs were dissolved in the release medium within 30 minutes in the eye drops group, and nearly 92% of the drug was released within 2 h. In contrast, release of BLZ from in situ gels in TF solution occurred after more than 12 h due to the sustained release mechanism of drug-polymeric compound. The complex exchanged with endogenous eye ions and delivered drugs at a controlled rate over a given period of time. Gelation reduced the rate of diffusion and erosion of polymers and associated drugs, thereby enhancing drug retention and bioavailability. These results indicate that the in situ gel retained the drug and could be used in the ocular administration systems. When the drug concentration was fixed, the higher the concentration of GG was, the lower the rate of drug release was. The release rates of various GG preparations can be divided into: 0.25%<0.5%<1.0%.

To investigate the drug-release mechanism, the release data were fit to models representing zero-order, first-order, Higuchi, and Ritger–Peppas equations. The linear regression analyses are summarized in Table 5. The coefficient of

Table 5 In vitro drug-release kinetics of BLZ from in situ gel system

Model	Equation	R^2
Zero-order	$y = 3.219x + 2.381$	0.8213
First-order	$y = -0.726x + 2.019$	0.9182
Higuchi	$y = 7.938x - 2.209$	0.8531
Ritger–Peppas	$y = 14.293x^{0.2716}$	0.8422

Abbreviation: BLZ, brinzolamide.

Table 6 Draize test scores

Location	Normal saline		Azopt		BLZ solution		BLZ in situ gel	
	Single	Long-term	Single	Long-term	Single	Long-term	Single	Long-term
Cornea	0	0	0	0	0	0	0	0
Iris	0	0	0.3	0	0	0	0	0
Conjunctival congestion	0	0	0	1	1	1	0.3	0.7
Conjunctival edema	0	0.7	0.3	0.3	0	1	0	0.3
Secretions	0	0	0	0	0	0	0	0
Total score	0	0.7	0.6	1.3	1	2	0.3	1

Abbreviation: BLZ, brinzolamide.

determination (R^2) values for the in situ gel indicated that the first-order equation was suitable for its release mechanism. But the release of the drug was significantly affected by gel dissolution after gel formation. Similar results were obtained by other investigators using different gelled systems.[24,25]

In vivo rabbit irritation test

The Draize method was used to evaluate the eye irritation of BLZ eye drops and in situ gel, with saline and Azopt preparations as control experiments. For all formulations, the corneal and iris scores were zero (Table 6). Although conjunctival hyperemia was observed in the group of BLZ in situ gel, there was no significant difference between normal saline and Azopt. Conjunctival hyperemia led to conjunctival sensitivity to exogenous compounds. The total scores of all formulations were valued between 0 and 3 in a single-dose or long-term eye irritation test. These results showed that BLZ in situ gel did not stimulate irritation of rabbit eye tissues and was less irritating than BLZ solutions and commercial Azopt.

Histological analysis of the corneal sections of different formulations after long-term irritation is shown in Figure 4.

As can be seen from Figure 4A, satisfactory epithelium and stroma structure with a little edema was maintained after the administration of normal saline. After long-term irritation tests, the corneal epithelial cells of eyes treated with BLZ in situ gel exhibited some slight edema (Figure 4B). However, there was no significant difference between the two groups ($p>0.05$).

Pharmacodynamic studies

Figure 5 indicates the change of IOP in the three groups of rabbits (normal saline, BLZ solution, and BLZ in situ gel) to determine the curative effect of treatment. In general, elevated IOP was observed in rabbits placed in a dark room.[26]

After 5 h in the dark, IOP was increased by 4–5.5 mmHg compared with untreated rabbits (15.03 mmHg). As can be seen from Figure 5, BLZ solution and BLZ in situ gel significantly hindered the increase in IOP compared with saline. Interestingly, however, IOP reduction in the BLZ in situ gel group was greater than in the BLZ solution group ($p<0.05$). It can be observed from the curve that the IOP of BLZ solution significantly declined by 27%

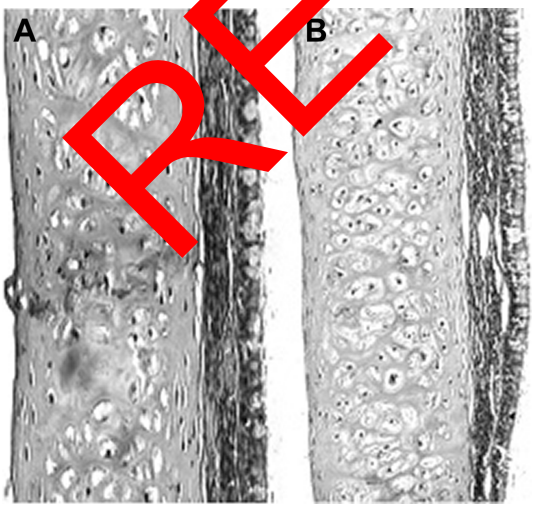

Figure 4 Cornea histopathology by microscopy.
Note: (**A**) Treated with saline, (**B**) treated with BLZ in situ gel.
Abbreviation: BLZ, brinzolamide.

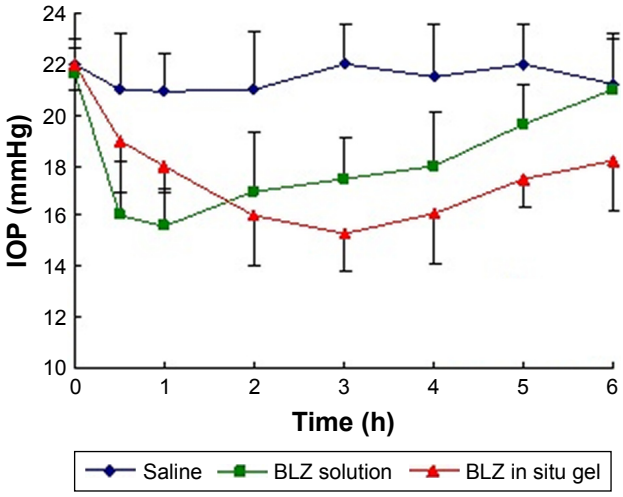

Figure 5 Change in IOP for rabbits with saline, BLZ solution, and BLZ in situ gel (n=4).
Abbreviations: BLZ, brinzolamide; IOP, intraocular pressure.

after 1 h but quickly recovered to baseline values after 6 h (21.2 mmHg). In contrast, IOP of BLZ in situ gel decreased slightly by 18.2% after 1 h before slowly increased to 18.6 mmHg below baseline values after 6 h. This implied that the novel preparation of BLZ in situ gel effectively prolonged the IOP-lowering effect after administration.

Conclusion

In this study, an eye delivery system was developed using an in situ gel carrier and appropriate doses of BLZ. The administration in the eyes of in situ gels containing GG proved to be safe and bioadhesive. With the presence of simulated tear solutions, in situ gelled vehicles were able to form a strong gel following the phase transition, allowing controlled drug release. Finally, in situ gel formation can be more effectively controlled and lead to a significant increase in the BLZ release.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Sun X, Lin M, Duan X, Zhang C, Ming J. Patient satisfaction with fixed-combination bimatoprost/timolol ophthalmic solution: a survey study in patients with glaucoma in China. *Patient Prefer Adherence*. 2[illegible]11: 845–852.
2. Actis AG, Dall'Orto L, Penna R, Brogliatti B, Rolle T. An in[illegible]rnal medicine perspective review of risk factors for as[illegible]sing and [illegible]gression of primary open angle glaucoma. *Miner[illegible]Med.* [illegible]13;104[illegible]: 471–485.
3. Nakano T, Inoue R, Kimura T, et al. Effects [illegible]brinzola[illegible]topical carbonic anhydrase inhibitor, on corne[illegible]endot[illegible]ells. *Adv Ther*. 2016;33(8):1452–1459.
4. Wang TH, Huang JY, Hung PT, Sh[illegible] Chen YF. Oc[illegible]r hypotensive effect and safety of brinzolam[illegible]e ophth[illegible]mic solution [illegible] open angle glaucoma patients. *J Formos Med Assoc*. 2[illegible]:103(5):369–373.
5. Osmałek T, Froelich A, [illegible]sarek S. Applicatio[illegible]f gellan gum in pharmacy and medicine. *[illegible] Pharm*. 2014;466(1–2):328–340.
6. Oliveira JT, Martin[illegible] Piccio[illegible]R, et al. Gellan gum: a new biomaterial for cartilage tissue [illegible]ering appli[illegible]ions. *J Biomed Mater Res A*. 2010;93:852[illegible]
7. Silva-Co[illegible]a J, C[illegible]eira JM[illegible]a[illegible]ade SG, et al. Gellan gum-based hydr[illegible]s for in[illegible]vertebral d[illegible]sc tissueengineering applications. *J Tissue Eng R[illegible]*;5(6):e97–e107.
8. Pahuja P[illegible]a S, Pawar P. Ocular drug delivery system: a reference to natural po[illegible]ers. *Expert Opin Drug Deliv*. 2012;9(7):837–861.
9. Stevens LR, Gilmore KJ, Wallace GG. Tissue engineering with gellan gum. *In Het Panhuis M Biomater Sci*. 2016;4(9):1276–1290.
10. Osmałek T, Milanowski B, Froelich A, et al. Design and characteristics of gellan gum beads for modified release of meloxicam. *Drug Dev Ind Pharm*. 2017;43(8):1–16.
11. Hoosain FG, Choonara YE, Kumar P, et al. In vivo evaluation of a PEO-gellan gum semi-interpenetrating polymer network for the oral delivery of sulpiride. *AAPS PharmSciTech*. 2017;18(3):654–670.
12. Morsi N, Ibrahim M, Refai H, El Sorogy H. Nanoemulsion-based electrolyte triggered in situ gel for ocular delivery of acetazolamide. *Eur J Pharm Sci*. 2017;104:302–314.
13. Reed K, Li A, Wilson B, Assamoi T. Enhancement of ocular in situ gelling properties of low acyl gellan gum by use of ion exchange. *J Ocul Pharmacol Ther*. 2016;32(9):574–582.
14. Zhu L, Ao J, Li P. A novel in situ gel b[illegible]f deacetylase gellan gum for sustained ophthalmic drug delive[illegible]of keto[illegible]: in vitro and in vivo evaluation. *Drug Des Devel The[illegible]*2015;9:3943–[illegible]9.
15. Salunke SR, Patil SB. Ion activa[illegible]in situ gel of g[illegible]an gum containing salbutamol sulphate fo[illegible]asal ad[illegible]istration. *I[illegible] Biol Macromol*. 2016;87:41–47.
16. Li X, Du L, Chen [illegible], et al. N[illegible]al deliv[illegible] analgesic ketorolac tromethamine therm[illegible]and io[illegible]ensitive in situ hydrogels. *Int J Pharm*. 2015;489(1–2[illegible]52–2[illegible]
17. Agrawal A[illegible] Das M, Jain[illegible] In situ[illegible] systems as 'smart' carriers for sustain[illegible]ar drug deliv[illegible]*pert Opin Drug Deliv*. 2012;9(4): 383–[illegible]2.
18. Wadhwa S, Paliw[illegible]R, Paliwal SR, Vyas SP. Nanocarriers in ocular [illegible]delivery: an u[illegible]te review. *Curr Pharm Des*. 2009;15(23): 2724–2750.
19. Dholakia M[illegible]Thakkar V, Patel N, Gandhi T. Development and characterisation of [illegible]ermos reversible mucoadhesive moxifloxacin hydrochloride in situ[illegible]h-thalmic gel. *J Pharm Bioallied Sci*. 2012;4:S42–S45.
20. [illegible] M, Pierson K, Hanamaikai K, Santiago-Caban L, Muthappan V, Passi SF. Artificial tears potpourri: a literature review. *[illegible] Ophthalmol*. 2014;8:1419–1433.
21. Thimmasetty MK, Mandal S, Prabhushankar GL, Geetha MS. Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *Int J Pharm Investig*. 2012;2(2):78–82.
22. Nisha GS, Maithil P, Charyulu RN. Formulation and development of nasal in situ gel softriptans for antimigraine activity. *Int J Res Pharm Biomed Sci*. 2012;3(2):861–868.
23. Buda I, Budai P, Szabó R, Lehel J. In vitro eye corrosion study of agrochemicals on isolated chicken eye. *Commun Agric Appl Biol Sci*. 2013;78(2):177–181.
24. Wu H, Liu Z, Peng J, et al. Design and evaluation of baicalin-containing in situ pH-triggered gelling system for sustained ophthalmic drug delivery. *Int J Pharm*. 2011;410(1–2):31–40.
25. Li J, Liu H, Liu LL, Cai CN, Xin HX, Liu W. Design and evaluation of a brinzolamide drug-resin in situ thermosensitive gelling system for sustained ophthalmic drug delivery. *Chem Pharm Bull*. 2014; 62(10):1000–1008.
26. Nunes CD, Vaz PD, Fernandes AC, Ferreira P, Romão CC, Calhorda MJ. Loading and delivery of sertraline using inorganic micro and mesoporous materials. *Eur J Pharm Biopharm*. 2007;66(3):357–365.