Introduction

Thermo-setting gel is a vehicle for ophthalmic solutions to improve the bioavailability of ophthalmic solution preparations. This technology has been applied to Rysmon TG, which is a long-acting ophthalmic solution containing timolol maleate. Using this vehicle, the preparation is applied as a solution and is transformed into a gel in the conjunctival sac by the surface temperature of the eye, thus allowing prolonged retention of the drug covering the ocular surface. The main ingredient of thermo-setting gel is methylcellulose, which has the property of reversible sol–gel transformation at around 55°C. Addition of sodium citrate dehydrate and polyethylene glycol induces gelation at the ocular surface temperature of 32°–34°C. The original thermo-setting gel is clear in solution form but becomes opaque after changing to gel form. A new thermo-setting gel is being developed that retains its transparency even in gel form. As an early-phase study to examine whether this vehicle can be used as artificial vitreous, we produced a rabbit model and investigated the safety of an intravitreous injection of the vehicle and the changes after injection.

Materials and Methods

Experimental Animals

Male Japanese white rabbits (Saitama Experimental Animals Supply, Saitama, Japan) weighing 2 to 3kg were used. The study was approved by our Institutional Animal Ethics Committee. All animal experiments were conducted...
in compliance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and our institutional guidelines.

Thermo-Setting Gel

The thermo-setting gel for injection was prepared with methylcellulose as the main ingredient and was mixed with polyethylene glycol.\(^1\) By changing the ratio of these ingredients, the gelation speed, viscosity, and transmissivity after gelation can be changed. This thermo-setting gel has characteristics that increase the gelation speed and viscosity, lowering the transmissivity. For the present intravitreous injection, various thermo-setting gels were prepared by changing the ratio of the ingredients, and the optimal preparation, WTG-127 (Wakamoto Pharmaceutical, Tokyo, Japan) was used. WTG-127 gelates at the relatively low temperature of 36°C, which is almost equivalent to body temperature, and also retains transparency even upon gelation. Its properties are shown in Table 1. In this study, WTG-127 (WTG hereafter) was injected intravitreally into rabbit eyes to evaluate its safety. In addition to the colorless gel, a blue gel was also prepared by adding blue dextran (blue-WTG) to facilitate visual identification.

Intravitreous Injection Method

Vitrectomy was performed in one eye of a white rabbit by the following procedures. The rabbit was anesthetized by intramuscular injection of 1.3 ml of a mixture of ketamine 50 mg/ml and xylazine 20 mg/ml (4:1). Then full mydriasis was induced by instilling 0.5% phenylephrine hydrochloride and 1% atropine. After conjunctival incision, 20-gauge pars plana sclerotomy was carried out at two sites. At one sclerotomy, an infusion tube was sutured for perfusion of intraocular infusion fluid (BSS plus; Santen Pharmaceutical, Osaka, Japan). Through the other sclerotomy, core vitrectomy was performed with a vitreous cutter (23-gauge high-speed cutter; Bausch and Lomb Japan, Tokyo, Japan). After the vitreous was adequately excised, we injected 1 ml of WTG by a 27-gauge blunt needle. Then the two sclerotomies were closed with sutures to complete the procedures. Animals were excluded from analysis when a retinal tear occurred intraoperatively. WTG was used in ten eyes of ten rabbits, and blue-WTG was used in three eyes of three rabbits. The contralateral control eyes were not given ophthalmic solution or surgery.

Observation Methods

The ten eyes injected with WTG and the ten contralateral eyes were observed and intraocular pressure and the electroretinogram were evaluated on days 1, 3, 7, 14, and 28. After all observations and evaluations were completed on day 28, the animals were killed with an overdose of anesthetic and the 20 eyes were enucleated and examined. The three eyes injected with blue WTG and the three contralateral eyes were only observed, and finally these animals were killed and the eyes enucleated in the same manner as above.

Slit-Lamp Microscopy and Funduscopy

The anterior ocular segment, optic media, and ocular fundus were examined with a slit-lamp microscope and a funduscope. The conditions of the conjunctiva, cornea, anterior chamber, crystalline lens, vitreous, and retina were evaluated for the presence or absence of inflammation, opacity, hemorrhage, and complications.

Intraocular Pressure

After instilling 0.4% oxybuprocaine hydrochloride, intraocular pressure was measured using an appplanation tonometer.

Electroretinography

Electroretinograms (ERG) were obtained using a portable ERG system PE-200 (Tomey, Nagoya, Japan) equipped with a contact lens electrode and a ground electrode. Both eyes of a rabbit were instilled with 0.5% phenylephrine hydrochloride to obtain full mydriasis. The rabbit was anesthetized by intramuscular injection of 1.3 ml of a mixture of ketamine 50 mg/ml and xylazine 20 mg/ml (4:1). Contact lens electrodes (Kyoto Contact Lens, Kyoto, Japan) were placed on the corneas of both eyes. Topical methylcellulose (1%) was used as the conducting medium. The reference and ground electrodes were attached to the forehead and the trunk of the animal. After dark adaptation for 15 min, a white hemispheric dome was placed over the head to obtain equal intensity of illumination to both eyes. The light stimulator was set 20 cm above the eyes, and flashes of 0.2-s duration and 20-J intensity were used. The recorded waveforms were calculated automatically, and values of a-wave and b-wave amplitudes and latency were used in the analyses.

Table 1. The properties of WTG-127

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.0</td>
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<tr>
<td>Time to gelate(^a)</td>
<td>50 min</td>
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<tr>
<td>Specific gravity (10°C)</td>
<td>1.01984</td>
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<tr>
<td>(after gelation)</td>
<td>1.01871</td>
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<tr>
<td>Transmissivity(^b)</td>
<td>89.3%</td>
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\(^a\) After soaking in a hot-water bath at 37°C.
\(^b\) When transmissivity of water is taken as 100%.