Japanese Journal of Ophthalmology (2019) 63:289–296
https://doi.org/10.1007/s10384-019-00665-8

THE OFFICIAL INTERNATIONAL JOURNAL OF THE JAPANESE OPHTHALMOLOGICAL SOCIETY

LABORATORY INVESTIGATION

Stability, safety, and pharmacokinetics of ganciclovir eye drops prepared from ganciclovir for intravenous infusion

Naoki Okumura[1] · **Toshiyuki Tanaka**[1] · **Yuya Fukui**[1] · **Noriko Koizumi**[1]

Received: 11 September 2018 / Accepted: 25 February 2019 / Published online: 2 April 2019

Abstract

Purpose Hospital-prepared topical ganciclovir eye drops made from intravenous infusions are used to treat cytomegalovirus corneal endotheliitis. This study assessed the efficacy of these eye drops.

Study design Experimental study design.

Methods Ganciclovir solutions (0.5% and 1.0%) prepared by diluting DENOSINE® IV Infusion in saline were stored light-shielded at 4, 25, or 37°C for 12 weeks. Every two weeks during storage, macroscopic evaluation was conducted and ganciclovir concentrations were determined by high performance liquid chromatography. Ocular surface toxicity and corneal ganciclovir concentrations were evaluated following topical instillation of ganciclovir solutions in rabbits.

Results Ganciclovir solutions maintained transparency for 6 weeks, with precipitation appearing after 8 weeks. Ganciclovir concentrations were maintained at ~100% for 6 weeks at 4°C and 25°C and decreased gradually to 90% after 12 weeks. At 37°C, ganciclovir concentrations decreased linearly for 12 weeks. Rabbit eyes showed no ocular surface toxicity. Following instillation of 0.5% ganciclovir solution, endothelial ganciclovir concentrations were 28.0 μg/g at one hour and 4.3 μg/g at three hours.

Conclusions Ganciclovir eye drops seem to be safe and penetrate the corneal endothelium. The drug in eye drop form is chemically stable for up to 6 weeks. Eye drops' development for approval by regulatory authorities, especially with improved long-term stability, is anticipated.

Keywords Ganciclovir eye drops · Cytomegalovirus corneal endotheliitis · Stability · Pharmacokinetics

Introduction

Corneal endotheliitis was first characterized by Khodadoust and Attarzadeh in 1982 as featuring linear keratic precipitates similar to endothelial rejection, bilateral recurrent corneal edema, and mild iritis [1]. Although this was initially considered a presumed autoimmune response [1], involvement of viral infection has been suggested as a pathology for corneal endotheliitis due to its poor response to steroid therapy [2]. Potential causative organisms included herpes simplex virus (HSV) and varicella zoster virus (VZV), as DNA from these viruses was detected in the corneal endothelium, aqueous humor, and trabeculae in some patients with corneal endotheliitis [3–8]. However, many patients have been diagnosed with idiopathic corneal endotheliitis, without any viral DNA detected in aqueous humor and the patients did not respond to acyclovir therapy.

In 2006, we reported the first patient with unilateral corneal endotheliitis exhibiting linear keratic precipitates and coin-shaped lesions associated with positive cytomegalovirus (CMV) DNA in the aqueous humor, without any HSV or VZV DNA being detected [9]. Subsequently, multiple studies, especially from Asian countries, have accumulated evidence suggesting that CMV endotheliitis is a new clinical entity [10–21].

Systemic and topical administration of anti-CMV drugs, including ganciclovir and valganciclovir, proved efficient in the treatment of CMV endotheliitis [10–21]. However, while systemic drug use was approved for CMV infectious diseases, such as CMV retinitis in immunocompromised

Corresponding author: Noriko Koizumi

✉ Noriko Koizumi
nkoizumi@mail.doshisha.ac.jp

[1] Department of Biomedical Engineering, Faculty of Life and Medical Sciences, Doshisha University, Kyotanabe 610-0321, Japan

patients and CMV pneumonitis in bone marrow transplant recipients, no topical use of an anti-CMV drug has yet been approved by regulatory authorities. Therefore, ganciclovir for intravenous infusion (DENOSINE®) or 0.15 % ganciclovir gel (Virgan®), which are approved for HSV keratitis, are often prepared as eye drops for off-label use as topical therapy [10, 12, 14, 19–21]. In Japan, 0.15 % ganciclovir gel is not approved for HSV keratitis, so hospital-prepared ganciclovir eye drops are widely used for CMV endotheliitis as a combination therapy with systemic ganciclovir or as a prophylactic therapy [10, 12, 14, 19, 20]. However, the extensive and long-term use of hospital-prepared eye drops might raise concerns regarding the chemical stability and safety of ganciclovir in this form, given the increased number of cases classified as CMV endotheliitis and the increasing numbers of patients being treated in hospitals.

In the current study, we evaluated the in vitro stability of ganciclovir eye drops prepared from DENOSINE® 500 mg for I.V. Infusion, and examined the ocular surface safety of ganciclovir eye drops using a standardized ocular irritation test in rabbits. We also evaluated the penetration of ganciclovir into the corneal endothelium by measuring the intracorneal concentration of ganciclovir by high performance liquid chromatography (HPLC) after a single administration of ganciclovir eye drops in rabbits.

Materials and methods

Ethics statement

Rabbits were housed and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The rabbit experiments were performed at Doshisha University (Kyoto, Japan) according to the protocol approved by the University's Animal Care and Use Committee (Approval No. A18081).

In vitro stability test

Ganciclovir (DENOSINE® 500 mg for I.V Infusion, Mitsubishi Tanabe Pharma Co.) was used to prepare 0.5% and 1.0% solutions for use as eye drops. A 500 mg sample of ganciclovir was diluted in 10 ml sterile water in laminar flow hood, and then diluted in 50 ml to prepare 0.5% solution and in 100 ml sterile saline to prepare 1.0% solution. The osmotic pressures of the 0.5% and 1.0% ganciclovir solutions were determined with an osmometer (Fiske Model 210 Micro Osmometer, Advanced Instruments Inc.). Samples (5 ml) of the 0.5% and 1.0% ganciclovir solutions were stored in glass test tubes at 4, 25, or 37°C in a light-shielded condition for 12 weeks. The appearance, transparency, color, and precipitation of the ganciclovir solutions were investigated every 2 weeks. The appearance of ganciclovir solutions stored at room temperature without light shielding was also investigated. The concentrations of ganciclovir in the 0.5% and 1.0% solutions were determined every two weeks using the HPLC protocol described below. The pH changes occurring in the 0.5% and 1.0% ganciclovir solutions over time were determined every two weeks with a pH meter (LAQUA F-50, Horiba).

Ocular irritation and pharmacokinetics test in rabbits

For ocular irritation tests, 50 µl of 0.5% or 1.0% ganciclovir solution was instilled in eye drop form in the right eyes of 8 rabbits every 1 hour for a total of 10 times (n=4). The animals were monitored for discomfort and symptoms and signs in the conjunctiva, cornea, and lids by macroscopic examination according to the Test No. 405: Acute Eye Irritation/Corrosion, OECD Guidelines for the Testing of Chemicals (https://doi.org/10.1787/9789264185333-en. Accessed July 15, 2018). The pH of tears after instillation of 50 µl of 0.5% ganciclovir solution was evaluated in the right eyes of four rabbits; it was evaluated using pH test paper (universal pH test paper; ADVANTEC) after instillation durations of 0, 1, 3, 5, 30, and 60 minutes.

For pharmacokinetic tests, 50 µl of the 0.5% or 1.0% ganciclovir solutions was instilled in eye drop form in the right eyes of 12 rabbits (n=3 for each concentration, at 2 time points). The rabbits were euthanized by injection of a lethal dose of pentobarbital into the marginal ear vein at one and three hours after ganciclovir solution instillation. The corneas were separated into epithelium, stroma, and endothelium under a stereomicroscope, and all samples were weighed. The corneal specimens were immersed in HPLC mobile phase and ground using a stirrer (Three-One Motor BL3000, Shinto Scientific Co.), for use in HPLC analysis according to the protocol described below for determination of ganciclovir concentrations.

High performance liquid chromatography (HPLC)

HPLC analysis was performed on Prominence LC-20AT HPLC analysis system (Shimadzu Corp.) on an octadecyl silica column (Inertsil ODS-3V C18 (4.6 mm i.d × 250 mm, 5.0µm), GL Sciences Inc.). The mobile phase consisted of 0.025 M ammonium acetate containing 0.4 g sodium hexane sulfonate and acetonitrile (90:10, v/v). The mobile phase was filtered through a 0.45 µm membrane filter, degassed, and then pumped at 1.0 ml/min flow rate. The column was thermostated at 30°C. The ganciclovir was detected by UV absorbance at a wavelength of 254 nm and its concentration was determined using a standard curve.

Results

The 0.5% and 1.0% ganciclovir solutions prepared from DENOSINE® 500 mg for I.V Infusion were transparent, without any color or precipitation. The osmotic pressure of the 0.5% solution was 310.33±0.47 mOsm/kg and of the 1.0% solution, 334.33±0.47 mOsm/kg, while that of saline was 286±0.26 mOsm/kg (n=3). The appearance of the 0.5% and 1.0% solutions did not change during 2, 4, and 6 weeks of observation at any temperature. However, the 0.5% ganciclovir solution preserved at 4°C exhibited a white precipitate after 8 weeks, while no apparent precipitation was observed at 25°C and 37°C in the light-shielded condition. The 1.0% ganciclovir solution showed a white precipitate at 4°C and 25°C after 8 weeks, while no apparent precipitation was observed at 37°C in the light-shielded condition. After 8 weeks of storage at room temperature without light shielding, both the 0.5% and 1.0% ganciclovir solutions showed precipitated crystals (Fig. 1a). After 12 weeks in the light-shielded condition, the 0.5% ganciclovir solution exhibited a white precipitate at 4°C and 25°C, while no apparent precipitation was observed at 37°C. The 1.0% ganciclovir solution exhibited a more evident white precipitate at 4°C and 25°C than was observed for the 0.5% ganciclovir solution after 12 weeks, but it retained its transparency, with no apparent precipitation, at 37°C in the light-shielded condition. After 12 weeks of storage at room temperature without light shielding, both the 0.5% and 1.0% ganciclovir solutions showed more evident crystal precipitation than was observed after 8 weeks (Fig. 1b).

Representative HPLC traces for the 0.5% ganciclovir solution showed a peak of ganciclovir (Fig. 2a). The concentration of the 0.5% ganciclovir solution was maintained at almost 100% for 6 weeks at 4°C and 25°C and then it decreased gradually to 90% after 12 weeks. By contrast, the concentration decreased linearly to 80% over 12 weeks when the 0.5% solution was stored at 37°C (Fig. 2b). The concentration of the 1.0% ganciclovir solution was maintained at almost 100% at 4°C for 6 weeks and decreased gradually to 90% after 12 weeks. However, at higher temperatures, the concentration of the 1.0% ganciclovir solution decreased throughout the 12 weeks, decreasing to 85% at 25°C and 70% at 37°C at 6 weeks, and to 78% at 25°C and 66% at 37°C at 12 weeks (Fig. 2c). The pH values for the 0.5% and 1.0% ganciclovir solutions immediately after preparation were 10.7 and 10.8, respectively. After 12 weeks, the pH values of the 0.5% ganciclovir solution decreased to 10.1 at 4°C, 9.3 at 25°C, and 9.2 at 37°C (Fig. 2d), and the pH values of the 1.0% ganciclovir solution decreased to 10.5 at 4°C, 9.8 at 25°C, and 9.5 at 37°C (Fig. 2e).

Representative anterior segment images are shown for the pre- and post- ocular irritation tests, following instillation of 50 µl of 0.5% ganciclovir solution every 1 hour for 10 times (Fig. 3a). Bright field and fluorescence staining images obtained by slitlamp microscopy showed no evident changes in the anterior segment, including the cornea, iris,

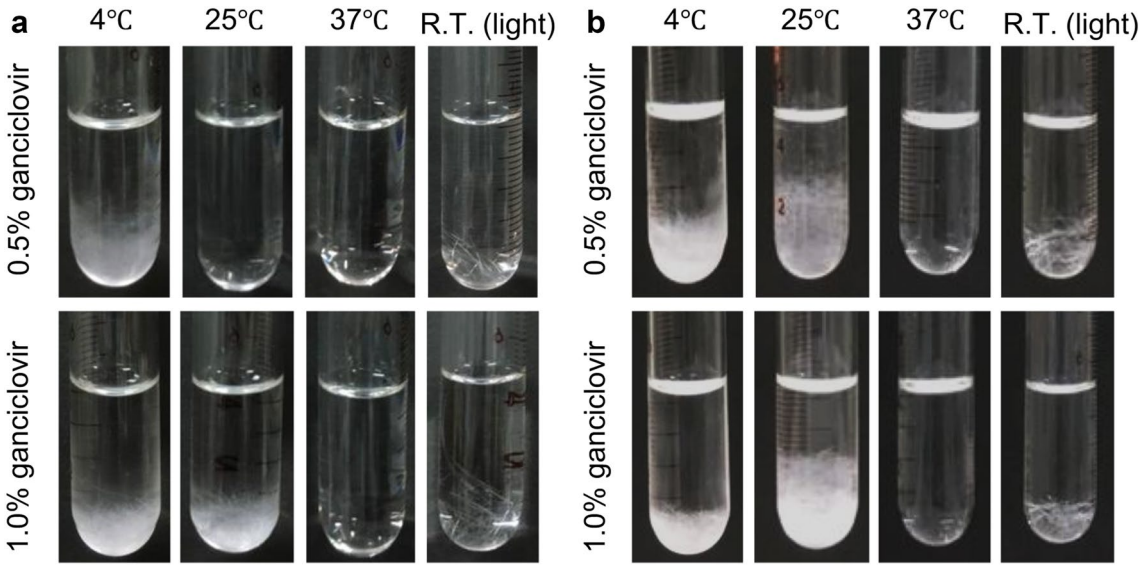

Fig. 1 Appearance test of 0.5% and 1.0% ganciclovir solutions. A 500 mg amount of ganciclovir (commercially available as DENOSINE® 500 mg for I.V Infusion, Mitsubishi Tanabe Pharma Co., Osaka, Japan) was diluted in 10 ml sterile water, and then diluted in 50 ml and 100 ml sterile saline to prepare 0.5% and 1.0% solution, respectively. Samples (5 ml) of ganciclovir solutions were stored in glass test tubes at 4, 25, or 37°C in a light-shielded condition for 12 weeks. The appearance of ganciclovir solutions stored at room temperature without light shielding was also investigated. Representative images of samples stored for 8 weeks (a) and 12 weeks (b) ware shown

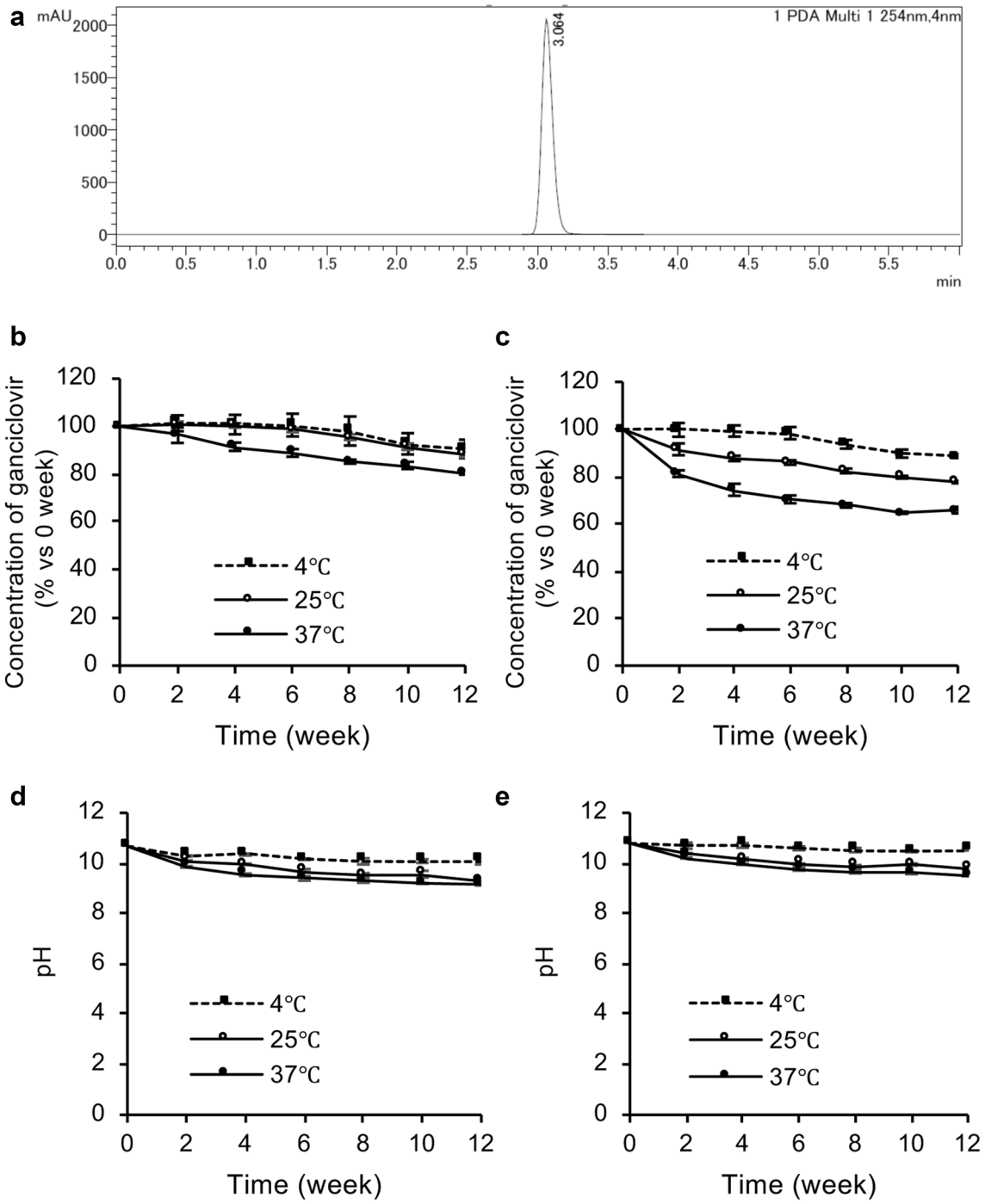

Fig. 2 Stability of ganciclovir concentration and pH in 0.5% and 1.0% ganciclovir solutions. (a) Ganciclovir concentrations of 0.5% and 1.0% ganciclovir solutions prepared from ganciclovir (commercially available as DENOSINE® 500 mg for I.V Infusion, Mitsubishi Tanabe Pharma Co., Osaka, Japan) were determined by high performance liquid chromatography (HPLC). Representative HPLC trace shows the peak of ganciclovir in the 0.5% ganciclovir solution. (b) Samples (5 ml) of 0.5% and 1.0% ganciclovir solutions were stored in glass test tubes at 4, 25, or 37°C in a light-shielded condition for 12 weeks. HPLC showed that concentration of 0.5% ganciclovir solution was maintained at almost 100% for 6 weeks at 4°C and 25°C and decreased gradually to 90% after 12 weeks, while it decreased throughout 12 weeks in a linear fashion to 80% after 12 weeks. (c) Concentration of 1.0% ganciclovir solution was almost 100% at 4°C and decreased gradually to 90% after 12 weeks. The 1.0% ganciclovir solution stored at 25°C or 37°C decreased in concentration gradually throughout 12 weeks, showing 85% at 25°C and 70% at 37°C at 6 weeks, and 78% at 25°C and 66% at 37°C at 12 weeks. (d)The pH of the 0.5% ganciclovir solution decreased to 10.1 at 4°C, 9.3 at 25°C and 9.2 at 37°C after 12 weeks. (e) pH of 1.0% ganciclovir solution decreased to 10.5 at 4°C, 9.8 at 25°C and 9.5 at 37°C after 12 weeks

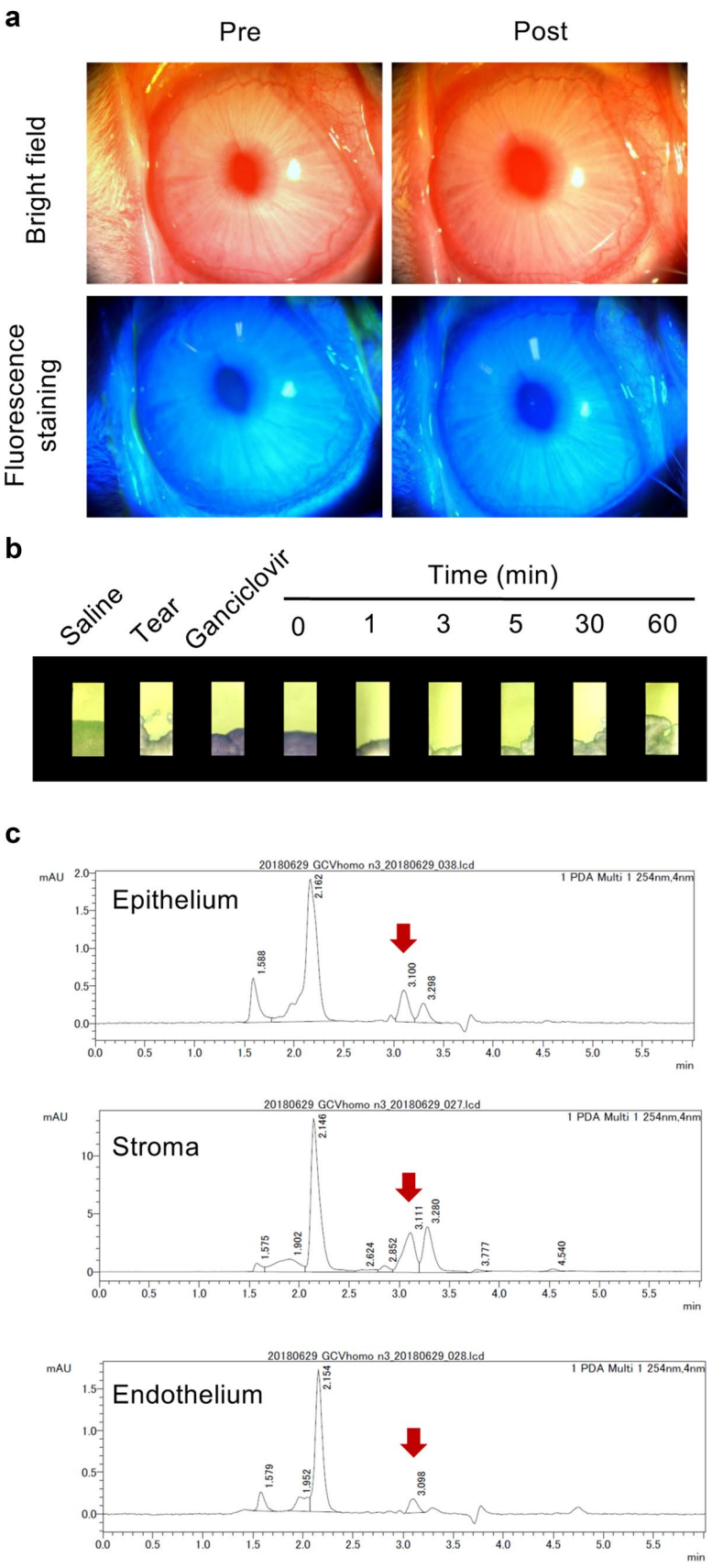

Fig. 3 Ocular irritation and pharmacokinetics tests in rabbits. (a) As an ocular irritation test, 50 µl of 0.5% and 1.0% ganciclovir solutions were instilled in rabbit eyes every 1 hour for 10 times. Representative bright field (upper) and fluorescence (lower) images of pre- and post- ocular irritation test obtained by slitlamp microscope are shown. n=3. (b) The pH of rabbit tears after instillation of 0.5% ganciclovir solution was evaluated using pH paper. Representative images showed that pH paper indicated a similar pH of rabbit tears and the ganciclovir solution immediately after instillation. The pH of tears decreased within one minute and became normal after 3 minutes. Experiments were performed in triplicate in independent rabbits. (c) A 0.5% and 1.0% ganciclovir solutions were instilled in rabbit eyes and concentrations of ganciclovir in corneas were determined by high performance liquid chromatography (HPLC). Representative results obtained by HPCL show the presence of ganciclovir in the corneal epithelium, stroma, and endothelium. Red arrows indicate the peak of ganciclovir

and conjunctiva. Evaluation using Test No. 405: Acute Eye Irritation/Corrosion, OECD Guidelines for the Testing of Chemicals, revealed no ocular discomfort or damage or severe alteration to the conjunctiva, cornea, and lids. Ganciclovir is an alkaline drug with a high pH, so we evaluated pH changes after instillation into the rabbit eye. The pH value for the 0.5% ganciclovir solution was approximately 9–10 at the time of instillation; however, the pH value of the tears decreased within one minute and became neutral after 3 minutes (Fig. 3b).

We also administered 0.5% and 1.0% ganciclovir solutions in rabbit eyes to evaluate the ganciclovir pharmacokinetics. Representative results obtained by HPLC confirmed the presence of ganciclovir in the corneal epithelium, stroma, and endothelium, as shown in Fig.3C. The concentrations of ganciclovir in the endothelium were 28.0 μg/g at one hour and 4.3 μg/g at three hours after instillation of 0.5% ganciclovir solution, and 56.3 μg/g at one hour and 5.3 μg/g at three hours after instillation of 1.0% ganciclovir solution (Table 1).

Discussion

Since our first case report of CMV corneal endotheliitis [9], several groups have detected CMV DNA in the aqueous humor of patients with corneal endotheliitis and reported that those patients responded to anti-CMV treatment [10–21]. In vivo laser confocal microscopy has shown that the corneal endothelial cells of patients with CMV endotheliitis exhibit a high reflection area surrounded by a halo of low reflection (owl's eye morphological features) [16]. This owl's eye morphological feature is generally observed in CMV infection in kidneys, lungs, and other organs [22], supporting CMV infection of the corneal endothelium [16]. In addition, an in vitro study has demonstrates that cultured human corneal endothelial cells efficiently support CMV replication [23]. This accumulating evidence shows that CMV is a causative pathogen of certain cases of "idiopathic corneal endotheliitis" and that CMV endotheliitis is a new clinical entity.

Table 1 Concentration of ganciclovir in corneal epithelium, stroma, and endothelium after instillation of ganciclovir solutions in rabbit eyes

Time (hour)	0.5% ganciclovir		1.0% ganciclovir	
	1	3	1	3
Epithelium	31.3±1.0	4.3±1.0	58.3±4.2	8.3±1.7
Stroma	886.7±38.6	59.7±2.1	1227.0±130.3	140.0±35.6
Endothelium	28.0±4.3	4.3±2.1	56.3±7.7	5.3±2.5

Data were shown as average ± standard deviation (μg/g)

A national survey of CMV endotheliitis in Japan conducted by the Japan Corneal Endotheliitis Study Group (JCESG) demonstrated clinical manifestations in 109 eyes of 106 patients with CMV endotheliitis [19]. Systemic and topical anti-CMV drugs, used singly or in combination, were tested in 104 of the 109 eyes (95.4%). Seventy-four eyes (67.9%) received systemic drugs (ganciclovir or valganciclovir) and 82 eyes (75.2%) received ganciclovir eye drops prepared from ganciclovir systemic drops. The combined use of systemic and topical anti-CMV drugs tends to be more effective than either systemic or topical treatments alone [19]. Future randomized studies are still necessary, while systemic as well as topical therapies are currently recognized as beneficial. Topical administration of ganciclovir eye drops is used as a combination therapy with systemic treatment and a prophylactic therapy for long-term use [20].

Hospital-prepared ganciclovir eye drops made from the ganciclovir systemic drops or 0.15 % ganciclovir gel (approved for HSV keratitis) can effectively treat CMV endotheliitis [10, 12, 14, 19–21]. As the 0.15 % ganciclovir gel is not approved in Japan, 0.5% ganciclovir eye drops'solution is widely used in Japan for clinical research under approval by institutional review boards. [10, 12, 14, 19, 20]. Here, we showed that the appearance of 0.5% and 1.0% ganciclovir eye drops remained unchanged during storage for 6 weeks, but white precipitation or crystals appeared after 8 weeks. The 0.5% ganciclovir solution maintained its concentration at almost 100% for 6 weeks at 4°C and 25°C, but the concentration decreased linearly at 37°C. These data suggest that the 0.5% ganciclovir eye drops are stable for 6 weeks and that storage in a refrigerator should be encouraged. The pH of the 0.5% ganciclovir solution was stable at 4°C, but its pH is higher than the usually approved pH for eye drops. However, our ocular irritation tests conducted in rabbits revealed no severe irritation or adverse effects. Interestingly, the pH of tears increased immediately after administration and then become neutral within 3 minutes. These pH changes probably reflect the limited buffering capacity of the ganciclovir solution, enabling the tears to reduce the pH immediately.

The ED50 of ganciclovir for CMV replication is reported as 100–1600 ng/ml [24]. The concentration of ganciclovir in the aqueous humor following administration of 0.15 % ganciclovir gel in human patients varied widely among individuals: we reported that it ranged from 24.3 to 691.0 ng/ml (average: 162.0±202.4 ng/ml) [20], while Waduthantri and colleagues recently reported a range of 0.67 to 33.53 ng/ml (average: 17.4±30.6 ng/ml) [21]. To the best of our knowledge, no report has yet described the concentration of ganciclovir in patients administered hospital-prepared ganciclovir eye drops. In the current study, we showed that following instillation of 0.5% ganciclovir solution in rabbit model the concentration of ganciclovir

in the corneal endothelium was 28.0±4.3 μg/g at one hour and 4.3±2.1 μg/g at three hour. Our current findings indicate that the concentration of ganciclovir in rabbit corneas was higher in the corneal endothelium than the ED50 level, which provides additional pharmacokinetics' data supporting the clinical observation that the 0.5% ganciclovir eye drops are beneficial for treatment or prophylaxis [10, 12, 14, 19, 20]. However, future development of anti-CMV eye drops that optimizes the concentration, frequency of application, and duration of drug use is anticipated.

One potential risk of hospital-prepared eye drops is microbial contamination. Multiple studies report various rates of microbial contamination of eye drops [25–31]. For instance, 64 (29%) of 220 eye drop preparations used for the treatment of ocular diseases tested positive for microorganisms [25]. In an extended care facility, 10 (8%) of 123 multi-dose solutions were contaminated with bacteria, though only 30% of the contaminated solution bottles were classified as dirty [30]. In terms of preservative free eye drops, 8 (8%) of 95 multiple application containers were contaminated [29]. Conversely, no contamination (0%) was observed in 49 preservative-free antibiotic eye drops (or eye drops containing diluted preservatives) [31]. A limitation of our current study is that we did not investigate the possible contamination of our eye drop preparation. The sterility of hospital-prepared ganciclovir eye drops used by patients must be confirmed in future studies.

In conclusion, we showed that both 0.5% and 1.0% ganciclovir eye drops prepared from ganciclovir systemic drops and stored light-shielded at 4°C and 25°C were stable in appearance, concentration, and pH for 6 weeks when. Our data support the safety and efficacy of the ganciclovir eye drops in current use in patients, but approval of topical ganciclovir drugs by regulatory authorities is needed. The issues of limited shelf stability, high pH, and the potential risk of microbial contamination of ganciclovir in the corneal endothelium await future development by pharmaceutical companies.

Acknowledgements The authors thank Mr. Hirofumi Imai and Mr. Tetta Kurosawa for technical support. The authors thank Scribendi.com for their professional English editing service. This study was supported by the Program for the Strategic Research Foundation at Private Universities from MEXT (Koizumi N and Okumura N).

Conflicts of interest N. Okumura, None; T. Tanaka, None; Y. Fukui, None; N. Koizumi, None.

References

1. Khodadoust AA, Attarzadeh A. Presumed autoimmune corneal endotheliopathy. Am J Ophthalmol. 1982;93:718–22.
2. Ohashi Y, Kinoshita S, Mano T, Kiritoshi A, Ohji M. Idiopathic corneal endotheliopathy. A report of two cases. Arch Ophthalmol. 1985;103:1666–8.
3. Maudgal PC, Missotten L, De Clercq E, Descamps J. Varicella-zoster virus in the human corneal endothelium: a case report. Bull Soc Belge Ophtalmol. 1980;190:71–86.
4. Sutcliffe E, Baum J. Acute idiopathic corneal endotheliitis. Trans Am Ophthalmol Soc. 1983;81:86–96.
5. Robin JB, Steigner JB, Kaufman HE. Progressive herpetic corneal endotheliitis. Am J Ophthalmol. 1985;100:336–7.
6. Ohashi Y, Yamamoto S, Nishida K, Okamoto S, Kinoshita S, Hayashi K, Manabe R. Demonstration of herpes simplex virus DNA in idiopathic corneal endotheliopathy. Am J Ophthalmol. 1991;112:419–23.
7. Amano S, Oshika T, Kaji Y, Numaga J, Matsubara M, Araie M. Herpes simplex virus in the trabeculum of an eye with corneal endotheliitis. Am J Ophthalmol. 1999;127:721–2.
8. Mimura T, Amano S, Nagahara M, Oshika T, Tsushima K, Nakanishi N, et al. Corneal endotheliitis and idiopathic sudden sensorineural hearing loss. Am J Ophthalmol. 2002;133:699–700.
9. Koizumi N, Yamasaki K, Kawasaki S, Sotozono C, Inatomi T, Mochida C, et al. Cytomegalovirus in aqueous humor from an eye with corneal endotheliitis. Am J Ophthalmol. 2006;141:564–5.
10. Suzuki T, Hara Y, Uno T, Ohashi Y. DNA of cytomegalovirus detected by PCR in aqueous of patient with corneal endotheliitis after penetrating keratoplasty. Cornea. 2007;26:370–2.
11. Chee SP, Bacsal K, Jap A, Se-Thoe SY, Cheng CL, Tan BH. Corneal endotheliitis associated with evidence of cytomegalovirus infection. Ophthalmology. 2007;114:798–803.
12. Koizumi N, Suzuki T, Uno T, Chihara H, Shiraishi A, Hara Y, et al. Cytomegalovirus as an etiologic factor in corneal endotheliitis. Ophthalmology. 2008;115(292–7):e293.
13. Anshu A, Chee SP, Mehta JS, Tan DT. Cytomegalovirus endotheliitis in Descemet's stripping endothelial keratoplasty. Ophthalmology. 2009;116:624–30.
14. Kandori M, Inoue T, Takamatsu F, Kojima Y, Hori Y, Maeda N, et al. Prevalence and features of keratitis with quantitative polymerase chain reaction positive for cytomegalovirus. Ophthalmology. 2010;117:216–22.
15. Chee SP, Jap A. Immune ring formation associated with cytomegalovirus endotheliitis. Am J Ophthalmol. 2011;152(449–53):e441.
16. Kobayashi A, Yokogawa H, Higashide T, Nitta K, Sugiyama K. Clinical significance of owl eye morphologic features by in vivo laser confocal microscopy in patients with cytomegalovirus corneal endotheliitis. Am J Ophthalmol. 2012;153:445–53.
17. Chu HY, Sun CC, Chuang WY, Liou SW, Ma DH, Lai CC, et al. Cytomegalovirus associated corneal endotheliitis after penetrating keratoplasty in a patient with Fuchs corneal endothelial dystrophy. Br J Ophthalmol. 2012;96:300–1.
18. Chee SP, Jap A. Treatment outcome and risk factors for visual loss in Cytomegalovirus endotheliitis. Graefes Arch Clin Exp Ophthalmol. 2012;250:383–9.
19. Koizumi N, Inatomi T, Suzuki T, Shiraishi A, Ohashi Y, Kandori M, et al. Clinical features and management of cytomegalovirus corneal endotheliitis: analysis of 106 cases from the Japan corneal endotheliitis study. Br J Ophthalmol. 2015;99:54–8.
20. Koizumi N, Miyazaki D, Inoue T, Ohtani F, Kandori-Inoue M, Inatomi T, et al. The effect of topical application of 0.15% ganciclovir gel on cytomegalovirus corneal endotheliitis. Br J Ophthalmol. 2017;101:114–9.
21. Waduthantri S, Zhou L, Chee SP. Intra-cameral level of ganciclovir gel, 0.15% following topical application for cytomegalovirus anterior segment infection: a pilot study. PLoS One. 2018;13:e0191850.
22. Herriot R, Gray ES. Images in clinical medicine. Owl's-eye cells. N Engl J Med. 1994;331:649.

23. Hosogai M, Shima N, Nakatani Y, Inoue T, Iso T, Yokoo H, et al. Analysis of human cytomegalovirus replication in primary cultured human corneal endothelial cells. Br J Ophthalmol. 2015;99:1583–90.
24. Field AK, Davies ME, DeWitt C, Perry HC, Liou R, et al. 9-([2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl)guanine: a selective inhibitor of herpes group virus replication. Proc Natl Acad Sci USA. 1983;80:4139–43.
25. Schein OD, Hibberd PL, Starck T, Baker AS, Kenyon KR. Microbial contamination of in-use ocular medications. Arch Ophthalmol. 1992;110:82–5.
26. Geyer O, Bottone EJ, Podos SM, Schumer RA, Asbell PA. Microbial contamination of medications used to treat glaucoma. Br J Ophthalmol. 1995;79:376–9.
27. Mason BL, Alfonso EC, Miller D. In-use study of potential bacterial contamination of ophthalmic moxifloxacin. J Cataract Refract Surg. 2005;31:1773–6.
28. Leite SC, de Castro RS, Alves M, Cunha DA, Correa ME, da Silveira LA, et al. Risk factors and characteristics of ocular complications, and efficacy of autologous serum tears after haematopoietic progenitor cell transplantation. Bone Marrow Transplant. 2006;38:223–7.
29. Rahman MQ, Tejwani D, Wilson JA, Butcher I, Ramaesh K. Microbial contamination of preservative free eye drops in multiple application containers. Br J Ophthalmol. 2006;90:139–41.
30. Jokl DH, Wormser GP, Nichols NS, Montecalvo MA, Karmen CL. Bacterial contamination of ophthalmic solutions used in an extended care facility. Br J Ophthalmol. 2007;91:1308–10.
31. Montes JA, Johnson D, Jorgensen J, McElmeel ML, Fulcher LC, Kiel JW. Potency and sterility of fortified tobramycin, fortified vancomycin, and moxifloxacin at 4, 24, and 35 degrees C for 14 days. Cornea. 2016;35:122–6.