

Preparation and Ocular Pharmacokinetics of Ganciclovir Liposomes

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ABSTRACT

Ophthalmic liposomes of ganciclovir (GCV) were prepared by the reverse phase evaporation method, and their ocular pharmacokinetics in albino rabbits were compared with those obtained after dosing with GCV solution. The *in vitro* transcorneal permeability of GCV liposomes was found to be 3.9-fold higher than that of the solution. After *in vivo* instillation in albino rabbits, no difference was found in the precorneal elimination rate of GCV from liposome vs solution dosing. The aqueous humor concentration-time profiles of both liposomes and solution were well described by 2-compartmental pharmacokinetics with first-order absorption. The area under the curve of the aqueous humor concentration-time profiles of GCV liposomes was found to be 1.7-fold higher than that of GCV solution. Ocular tissue distribution of GCV from liposomes was 2 to 10 times higher in the sclera, cornea, iris, lens, and vitreous humor when compared with those observed after solution dosing. These results suggested that liposomes may hold some promise in ocular GCV delivery.

KEYWORDS: Ganciclovir, liposomes, precorneal clearance, ocular pharmacokinetics

INTRODUCTION

Ganciclovir (GCV) exhibits antiviral activity against herpes simplex virus (HSV) and cytomegalovirus (CMV) at relatively low inhibitory concentrations (IC₅₀ of ~50 and 900 ng/mL, respectively).¹⁻⁴ Therefore, GCV plays an important role in the treatment of ocular HSV and CMV infection. Because of its low lipophilicity, GCV showed poor ocular availability when topically applied.^{5,6} Ophthalmic GCV solution had been marketed in China, but its indication was limited to treat infection of the cornea with HSV. The use of conventional ophthalmic solution of GCV to treat ocular CMV infection is quite limited, therefore it is worthwhile to consider alternate delivery systems of GCV for the treatment of ocular HSV and CMV infections.

Serious ocular CMV infections, such as those in the cornea, sclera, or retina can occur in several groups of patients with impaired immunity (immunocompromised hosts), such as those with acquired immunodeficiency syndrome (AIDS), those undergoing chemotherapy, or those receiving immunosuppressive drugs for bone marrow or organ transplantation. CMV retinitis usually begins in one eye but often progresses to the other eye. Without treatment, progressive retinal destruction will lead to blindness in 4 to 6 months. In most cases, an intravenous dose (10.0 mg/kg daily, 7 to 21 days) of GCV halts disease progression. Unfortunately, the disease recurs after discontinuation of the drug.⁷ A conventional treatment to prevent relapse of CMV retinitis involves oral administration of GCV at a dose of 3000 mg/d. Such a high dose results in adverse effects,⁸ such as dose-related bone marrow suppression and neutropenia.

To increase the vitreous concentration of GCV, 2 types of GCV ophthalmic implants have been developed as a local treatment option that avoids systemic side-effects.⁹ One type of implant is made of nonbiodegradable ethylene-vinyl acetate (EVA) copolymer and poly(vinyl alcohol) and releases GCV for 6 to 8 months by passive diffusion through a small opening in EVA at the base of the device. This device requires a surgical procedure for its removal or repeated implantation when drug release is completed, posing the risk of retinal detachment, endophthalmitis, or vitreous hemorrhage. Another type of implant is composed of blending poly(D,L-lactide) of 2 different molecular weights. The blended implants are promising for intraocular controlled drug delivery over a period of several months to 1 year to treat cytomegalovirus retinitis. However, this system also requires a surgical implantation procedure.

Recently, liposomes have received considerable attention as ocular drug delivery systems owing to their ability to enhance ocular drug absorption. Liposomes offer advantages over most ophthalmic delivery systems in making intimate contact with corneal and conjunctival surfaces, thereby increasing the probability of ocular drug absorption. Law et al¹⁰ demonstrated that cationic or anionic liposomes of acyclovir could delay the precorneal clearance, hence increasing the ocular availability of acyclovir. However, there are no reports on delivering drug to the vitreous humor by liposomes.

The objective of the present study, therefore, was to evaluate the ocular pharmacokinetics of GCV liposomes, and to

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investigate the possibility of delivering GCV to vitreous humor.

MATERIALS AND METHODS

Materials

Phosphatidylcholine (PC, Epikuron 200, batch No. 139038) was a gift from Degussa (Düsseldorf, Germany). Ganciclovir was obtained from Huayuan Pharmaceutical Co (Shanghai, China). GCV ophthalmic solution (1 mg/mL) was purchased from Wuhan Tiantianming Pharmaceutical Co (Wuhan, China). Methanol of high-performance liquid chromatography (HPLC) grade was purchased from Shandong Yuwang Sci-Tech Co, Ltd (Shandong, China) and cholesterol (CH) was purchased from Tai Wei Sci-Tech Co, Ltd (Shanghai, China). Sephadex G-50 and sodium deoxycholate (NaDC) were purchased from Sigma-Aldrich Co, Ltd (Stockholm, Sweden). Dialysis bags (molecular cut off >8000) were purchased from Wan Qing Sci-Tech Co, Ltd (Nanjing, China). Other chemicals were of analytical grade.

both male and female albino rabbits (purchased from Qinglongshan Experimental Animal Center, Nanjing, China) weighing 2 to ~2.5 kg were used throughout and were cared for in accordance with the Guide for the Care and Use of Laboratory Animals.¹¹

Preparation of GCV Liposomes

GCV liposomes were prepared by reverse phase evaporation (REV) developed by Szoka and Papahadjopoulos.^{12,13} In brief, 450 mg of PC/CH/Na deoxycholate mixture (12:1.7:1, wt/wt), dissolved in 30 mL of chloroform/diethyl ether (1:3, vol/vol), was mixed with 10 mL of GCV solution (1.2 mg/mL) and sonicated for 10 minutes using KQ-250 sonicator (Kunshan Sonicator Co, Jiangsu, China) to form an emulsion (water/oil, [w/o]). The GCV liposomes were obtained by evaporation of the organic solvents using a rotary evaporator under vacuum at 37°C, and 2 mL of saccharose solution (5% wt/vol) were added. Finally, the GCV liposome preparation (1.0 mg/mL) was obtained by filtration through 0.22- μ m microporous filter (Wan Qing Sci-Tech Co, Ltd, Nanjing, China) to obtain a more homogeneously sized vesicles population.¹⁴

Determination of GCV Encapsulation Efficiency

GCV encapsulation efficiency (EE%) was determined by size exclusion chromatography (SEC).¹³ In brief, 100 μ L of GCV liposome preparation were separated by Sephadex G-50, and 5% saccharose solution was used as the elution solution. Separation was monitored by determining the absorbance of the solution at 254 nm using 752C spectrophotometer (Shanghai Analytical Instrumental Factory,

Shanghai, China). The encapsulated GCV was determined at 254 nm by spectrophotometry after lysis of liposomes with Triton X-100 (final concentration 0.5% vol/vol). The GCV EE% was calculated as follows:

$$EE\% = (Me/Mt) \times 100\% \quad (1)$$

where *Me* and *Mt* represent the encapsulated GCV amount and total GCV amount, respectively.

Particle Size and Zeta Potential of Liposomes

Particle size of GCV liposomes was analyzed by light scattering measurements using a Zetasizer 3000HS particle size analysis system (Malvern Instruments Ltd, Malvern, UK) at 20°C. The zeta potential of liposomes was measured with the laser Doppler method (heterodyne method) by using a Zetasizer 3000HS (Particle Sizing System, Malvern) at pH 6.5.

In Vitro Transcorneal Experiments

The transcorneal experiment was performed with a conventional diffusion chamber. The receptor solution consisted of glutathione bicarbonate Ringer's solution (GBR), which was composed of 0.092 g/L glutathione, 2.454 g/L sodium bicarbonate, 0.115 g/L calcium chloride dihydrate, 0.358 g/L potassium chloride, 0.159 g/L magnesium hydrochloride pentahydrate, 0.103 g/L sodium dihydrogen phosphate, 6.2 g/L sodium chloride, and 0.9 g/L glucose and adjusted to pH 7.2. before use, the receptor solution was aerated with the mixture of 95% O₂ and 5% CO₂ to maintain oxygenation of cornea.

Albino rabbits were humanely killed by intravenous injection of excess sodium pentobarbital, and the whole eyes were enucleated. The corneas of both eyes were excised and then mounted on the diffusion chamber. A 5- mL aliquot of the receptor solution was added to the endothelial side, while 0.5 mL of the GCV liposome preparation or GCV solution was added to the epithelial side. The temperature in the diffusion chamber was maintained at 34°C \pm 0.5°C by a thermostatic water bath. The receptor buffer was removed at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 hours and immediately replaced by previously aerated fresh receptor buffer. The sample was filtered through a 0.45- μ m microporous membrane, and the filtrate was kept in 4°C until analyzed by HPLC.

The apparent permeation coefficient (*P_{app}*, cm/s) of GCV was determined by

$$P_{app} = \frac{\Delta Q}{\Delta t \cdot C_0^D \cdot A \cdot 3600}, \quad (2)$$

where *C₀^D* is the initial concentration of GCV in the donor compartment, and *A* is the area of the cornea. For the calculation of the apparent permeation coefficient in the present study,

A was determined as 0.78 cm^2 . $\Delta Q/\Delta t$ is the steady-state rate of drug permeation across the intact cornea, as obtained from the slope of the straight line relating corneal permeability to time.

Precorneal Clearance Study

Five rabbits were used to determine precorneal clearance. Each rabbit was instilled with 50 μL GCV liposome preparation (1 mg/mL) on the right eye, and 50 μL GCV ophthalmic solution (1 mg/mL) on the left eye, respectively. At 0, 5, 10, 15, 30, 45, and 60 minutes after instillation, tear samples of both eyes were collected by Schirmer test strips (Tianjin Jingming New-Tech Co, Tianjin, China). The amount of tear withdrawn was calculated by subtracting the weight of each strip after sampling from the weight before sampling. The Schirmer strip was then placed into an Eppendorf tube, dried by an N_2 stream, and then 0.2 mL of mobile phase was added. The sample was vortexed thoroughly to dissolve GCV into the mobile phase and centrifuged; 20 μL of the supernatant liquid was analyzed for GCV by HPLC.

In Vivo Instillation

Albino rabbits were divided into 8 groups of 5 rabbits each, and 50 μL of GCV liposome preparation (1 mg/mL) and GCV ophthalmic solution (1 mg/mL) were dropped into the conjunctival sac of each eye, liposomes in the right and GCV solution the left. At 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, and 48.0 hours after drug administration, each rabbit was injected with 20% urethane (1.0 g/kg) through the ear limbus vein, followed by 0.1% 0.1 mL tetracaine instillation into the eye. The surface of the eye was briefly washed with 10 mL 0.9% sodium chloride solution, and the excessive water was blotted with filter paper. An aliquot of 100 μL of the aqueous humor was aspirated from the anterior chamber by paracentesis using a 30-gauge needle attached to a 1-mL syringe. The aqueous humor samples were stored at -18°C for analysis.

To investigate the distribution in ocular tissues of GCV, rabbits were humanely killed, and their eyes were enucleated at 0.5, 2.0, and 4.0 hours after dosing. The eyes were carefully rinsed with normal saline and dried with filter paper to remove remaining drug. Corneal epithelium was carefully removed using a scalpel. The corneas were excised at the limbus with scissors. The central vitreous humor was aspirated using an 18-gauge needle attached to a 2-mL syringe before the lens and iris ciliary body were removed. The sclera was scraped to remove all adherent choroidal and retinal tissues. All tissues were thoroughly homogenized using a glass homogenizer and then transferred to preweighed tubes. All tubes were weighed again before samples were stored at -70°C before analysis.

For analysis, each sample was mixed with an equal volume of 0.2% (vol/vol) perchloric acid. After centrifuging, 20 μL of the supernatant liquid was obtained for HPLC analysis.

Determination of HPLC Analysis of GCV

The HPLC system consisted of a Waters 515 HPLC pump, Waters 2487 HPLC detector (Waters Corp, Milford, MA) set at 254 nm, and a Sanrui Chromatography Workstation (Sanrui Sci-Tech Co, Shanghai, China). The samples were chromatographed on a reversed-phase LiChrospher- C_{18} column (150 \times 4.6 mm, 5 μm , Jiangsu Hanbang SciTech Co, Huaiyin, China), and a 4 \times 10mm precolumn of the same material. The mobile phase, at 1.0 mL/min flow rate, consisted of acetonitrile/water (0.4:99.6, vol/vol), which was filtered and degassed before use. The column was thermostated at 30°C , and under these experimental conditions, the run time was 18 minutes.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic analysis were performed using the 3p87 Pharmacokinetic Program (Chinese Society of Mathematical Pharmacology 1987, China). Statistical comparisons were made using analysis of variance (ANOVA) or the Student t test, where appropriate, and statistical significance was set at $P < .05$.

RESULTS AND DISCUSSION

Characteristics of GCV Liposomes

GCV liposomes, prepared by reverse phase evaporation (REV) method, were examined by transmission electron microscopy using the negative staining method (Figure 1). Spherical liposomes could be seen and the particle size of GCV liposomes was determined as $210 \pm 17 \text{ nm}$ (poly index = 0.283 ± 0.015) using light scattering measurements. The zeta potential of liposomes was measured as $-52.4 \pm 6.8 \text{ mV}$ using the laser Doppler method. The results indicated that the liposomes prepared by the REV method were much smaller than those reported by Law and Hung,¹⁴ which were prepared by 2 other methods, namely, drug-lipid film hydration ($1286 \pm 868 \text{ nm}$) and lipid film hydration with drug solution ($1091 \pm 745 \text{ nm}$).

The EE% of GCV-containing liposome was determined as $51.2\% \pm 1.3\%$ by gel chromatography method. The results indicated that negatively charged GCV liposomes with particle size of $\sim 200 \text{ nm}$ were easily prepared by the REV method.

In Vitro Transcorneal Permeation

Figure 2 shows the in vitro transcorneal permeation profiles of GCV solution and GCV liposomes. A good linearity

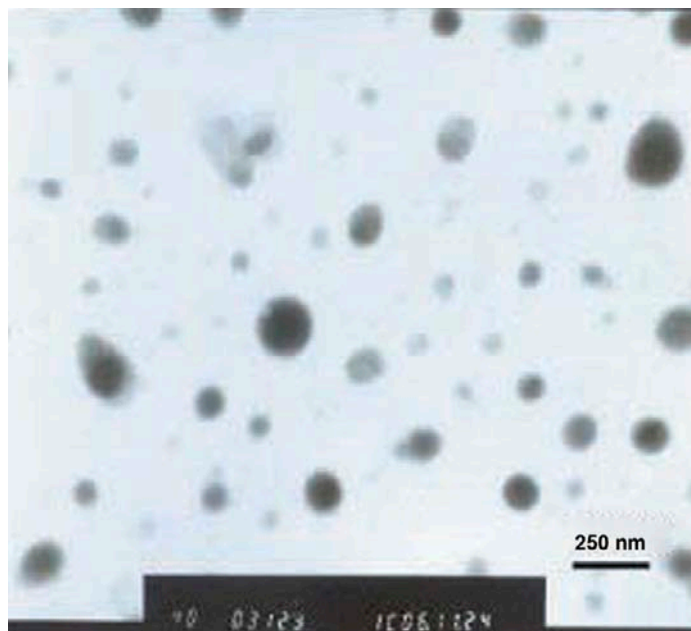


Figure 1. Negative-staining transmission electron microscopy images of liposomes. Magnification 1,000,000 \times .

between cumulative amount of GCV permeated through cornea and time was observed. The measured Papps of GCV were 3.93 ± 0.62 (GCV liposomes) and 1.01 ± 0.32 (GCV solution), respectively. GCV liposomes demonstrated a 3.9-fold higher permeability than that of GCV solution ($P < .05$). The results were different with that of acyclovir liposomes reported by Law et al,¹⁰ who reported that the permeability of acyclovir liposomes ($P_{app} = 1.44$) was lower than that of acyclovir solution ($P_{app} = 2.60$). This discrepancy could be explained by 2 possible reasons. First, the difference of Papps of GCV liposomes and

acyclovir liposomes might be because of a difference in the particle size. The smaller GCV liposomes (200 nm) could penetrate through the cornea more easily than the larger acyclovir liposomes (1000 nm). Second, the difference of Papps of GCV and acyclovir might also arise from the difference in their octanol-water partition coefficients (logPs). The logPs were calculated as -1.614 (acyclovir), and -2.165 (GCV) by Molinspiration (<http://www.molinspiration.com>). Acyclovir is therefore more lipophilic than ganciclovir, and because the hydrophobic nature of the upper epithelial layers of cornea made it a barrier for hydrophilic molecules, lipophilic molecules would display higher steady-state corneal permeability than hydrophilic molecules.¹⁵ As a result, the ocular permeability (Papp) of acyclovir solution would be expected to be higher than that of GCV solution (Figure 2).

Precorneal Clearance Study

Durrani et al¹⁵ established a bi-exponential equation to describe the precorneal clearance kinetics for ophthalmic hyaluronic acid:

$$y = A \times \exp(-k_1t) + B \times \exp(-k_2t), \quad (3)$$

where k_1 represents the initial clearance rate caused by tear drainage, and k_2 is a slow clearance phase caused by distribution process. Figure 3 shows the precorneal GCV concentration-time profiles of GCV liposomes and GCV solution. In both cases, the profiles (up to 20 minutes) were describable with the mono-exponential equation using SigmaPlot 9.0 software (Systat Software Inc, San Jose, CA):

$$y = A \times \exp(-k_e t), \quad (4)$$

where k_e represents the apparent clearance rate. This result suggested that for both GCV liposomes and GCV solution, GCV clearance was caused mainly by tear drainage and

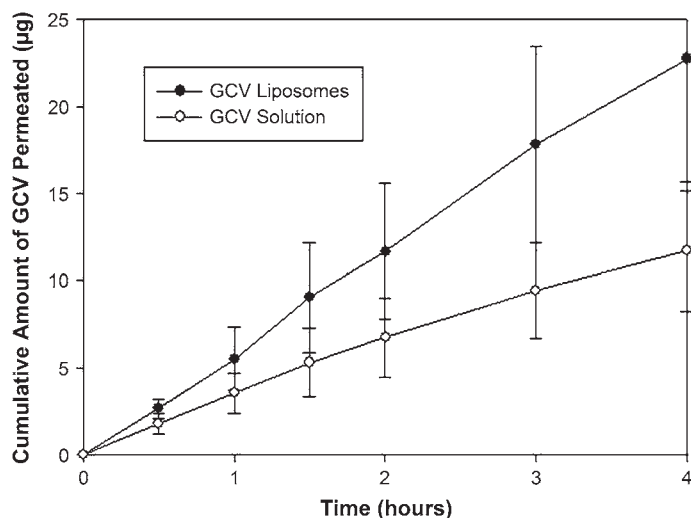


Figure 2. In vitro transcorneal permeation of GCV liposome preparation and solution ($\bar{X} \pm SD$, $n = 5$). GCV indicates ganciclovir.

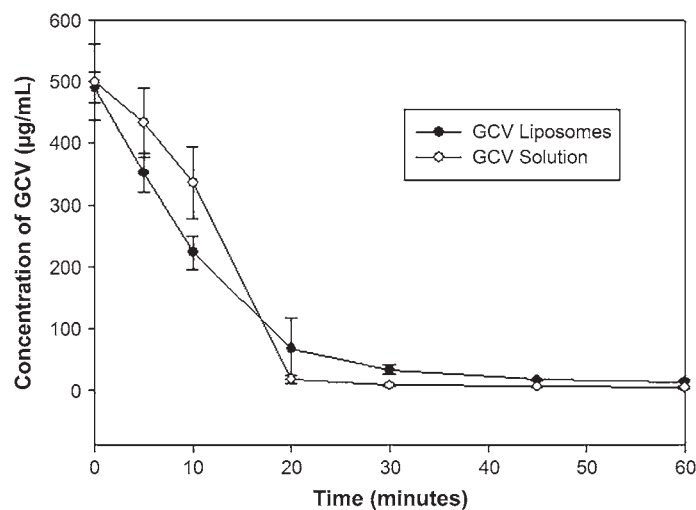


Figure 3. Kinetics of ganciclovir disappearance from tear fluid following topical administration of the liposome and the control solution ($\bar{X} \pm SD$, $n = 4$). GCV indicates ganciclovir.

turnover. Because both GCV preparations did not contain any viscous materials, it is possible that both could be eliminated from the precorneal area sufficiently rapidly so that the distribution process would not affect precorneal clearance.

Estimates of the kinetic parameters shown in Equation 4 resulted in values of A of $544 \pm 11 \mu\text{g/mL}$ (GCV liposomes) and $596 \pm 59 \mu\text{g/mL}$ (GCV solution), and k_c of $0.0923 \pm 0.0040 \text{ minute}^{-1}$ (GCV liposomes) and $0.0836 \pm 0.018 \text{ minute}^{-1}$ (GCV solution), respectively ($P > .05$, in both cases). This result indicated that the precorneal clearance of GCV liposome preparation was similar to that of GCV solution.

In Vivo Ocular Pharmacokinetics Study

The concentration-time profiles of GCV in aqueous humor after instillation of 1.0 mg/mL GCV liposome preparation and GCV solution in conscious rabbits are depicted in Figure 4. The concentration-time profiles of GCV of both liposomes and solution can be described by a 2-compartment model with first-order transcorneal absorption.

Table 1 summarizes the results of the compartmental analysis. The C_{max} and area under the curve (AUC) of GCV liposome preparation were significantly higher than that of GCV solution ($P < .001$). the absorption rate constant (K_a) of GCV liposome preparation was higher than that of GCV solution ($P < .05$). This finding was consistent with the higher transcorneal permeability of GCV liposome preparation than the GCV solution. However, it was shown that the mean residence time (MRT) of GCV liposomes was not significantly different from that of the GCV solution ($P > .05$).

The GCV concentrations in ocular tissues after instillation of GCV liposomes and GCV ophthalmic solution are com-

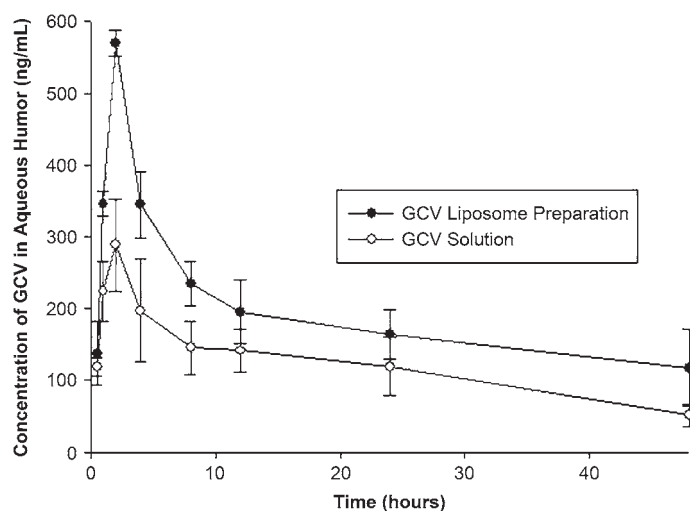


Figure 4. Concentration-time profiles of GCV in aqueous humor after instillation of 1.0 mg/mL GCV liposome preparation and GCV solution in rabbit (ng/mL, $\bar{X} \pm SD$, $n = 5$). GCV indicates ganciclovir.

pared in Table 2. The results indicate that the concentration of GCV of both dosage forms can be detected in aqueous humor, lens, and vitreous humor. In all ocular tissues, significantly higher drug concentrations at all time points were obtained after application of GCV liposomes when compared with solution dosing ($P < .05$). Liposomal incorporation of GCV increased the drug concentration by 2- to 10-fold in all these tissues. After application of GCV liposomes, the highest concentrations of GCV were found in the sclera and the cornea (1000-1500 ng/g), both of which are higher than the half maximal inhibitory concentration (IC_{50}) of GCV against CMV (900 ng/g).¹⁶

The concentrations of GCV in the aqueous humor, lens, and vitreous humors in the liposome-treated group were found to be within the range of 100 to 500 ng/g, which were several times higher than those of the solution-treated group (30-300 ng/g). Because over 70% of fluid in vitreous humor moves toward the retina and exits the vitreous cavity through the retina, GCV concentration in vitreous humor could be used to evaluate the possible effectiveness of GCV against CMV retinitis. These results would suggest the concentration attained with GCV liposomes was not sufficiently high enough for effectiveness in CMV retinitis.

CONCLUSIONS

GCV is an effective agent against ocular HSV and CMV infection. The main obstacle to its ophthalmic dosage forms is its low ocular bioavailability, especially its poor vitreous availability. In the current studies, ocular bioavailability of GCV liposomes in rabbits was shown to be 1.7-fold higher than that of GCV solution. This result was consistent with the observation that GCV liposomes demonstrated a 3.9-fold higher transcorneal permeability. The higher transcorneal permeability is consistent with interactions between liposomes and the corneal epithelial surface, thereby increasing the probability of drug penetration.¹⁷

In addition to increasing corneal drug absorption, there is evidence that liposomes may enhance intraocular GCV supply via the noncorneal route. Liposomes have, like other microparticulate systems, the tendency to accumulate in the conjunctival folds after drainage has subsided. It was suggested that conjunctival penetration is important in delivering liposomes.¹⁸ In the present studies, because of the difficulty of separating the conjunctiva from cornea and sclera, the conjunctiva was discarded, and the conjunctival GCV concentration was not monitored.

In the current study, GCV in liposomes showed significant trans-ocular absorption. The exact mechanisms are not clear, but particle size and the composition of GCV liposomes may play important roles. Small GCV liposomes (200 nm) have been shown to penetrate more readily through the cornea.

Table 1. Pharmacokinetic Parameters of GCV in the Aqueous Humor of Conscious Rabbits (n = 5)*

Pharmacokinetic Parameters	GCV Liposomes	GCV Solution
A (µg/mL)	4.200 ± 2.164†	0.427 ± 0.298
α (1/h)	0.763 ± 0.109§	0.236 ± 0.086
B (µg/mL)	0.254 ± 0.023†	0.420 ± 0.118
β (1/h)	0.0198 ± 0.0063†	0.0365 ± 0.0197
Ka (1/h)	1.57 ± 0.73†	2.88 ± 0.33
K ₂₁ (1/h)	0.121 ± 0.017†	0.251 ± 0.097
K ₁₀ (1/h)	0.165 ± 0.097†	0.0412 ± 0.0114
K ₁₂ (1/h)	0.699 ± 0.303‡	0.335 ± 0.158
Tmax (h)	1.96 ± 0.42	2.03 ± 0.36
Cmax (µg/mL)	0.570 ± 0.018§	0.312 ± 0.064
AUC (µg/mL)*hr	8.66 ± 1.18‡	4.87 ± 1.56
AUC (µg/mL)*hr _{0→∞}	11.0 ± 2.6†	7.11 ± 2.34
MRT(hours)	18.1 ± 2.0	17.6 ± 1.0
AUMC (µg*h ² /mL)*hour	158 ± 37†	101 ± 32
AUMC (µg*h ² /mL)*hour _{0→∞}	361 ± 176	288 ± 86

*GCV indicates ganciclovir; AUC, area under the curve; MRT, mean retention time; AUMC, area under the moment curve.

†Significantly different compared with GCV solution; *P* < .05.

‡Significantly different compared with GCV solution; *P* < .01.

§Significantly different compared with GCV solution; *P* < .001.

Another possible contributing factor may involve sodium deoxycholate, which may act as an edge activator to facilitate the formation of elastic liposomes. Elastic liposomes are deformable and can enter the stratum corneum carrying drug molecules into the skin.¹⁹ Because the corneal structure is similar to that of the stratum corneum, liposomes containing sodium deoxycholate may penetrate through the cornea and sclera more readily.

The highest GCV concentration was obtained in the sclera after liposome dosing. This result can be explained by the rapid distribution of GCV liposomes into sclera and trans-

scleral absorption. Based on the data listed in Table 2, it is likely that the sclera pathway may play an important role in increasing the ocular absorption of GCV liposomes. After application with GCV liposomes, the GCV concentrations in cornea and sclera (but not those in the aqueous humor, lens, iris, and vitreous humor) were higher than the IC₅₀ of GCV against CMV. Thus, GCV liposomes may be effective against CMV infection in the cornea and sclera. However, to treat CMV retinitis, further optimization of the formulation and administration regimen is needed.

Table 2. Concentration (µg/g) of GCV in the Ocular Tissues of Rabbits (n = 5) at Different Time Points After Administration of GCV Liposomes and Solution*

Tissue Sample		0.5 Hours	2 Hours	4 Hours
Cornea	Liposomes	0.70 ± 0.09†	0.96 ± 0.33†	0.66 ± 0.12
	Solution	0.48 ± 0.23	0.43 ± 0.18	0.32 ± 0.03
Sclera	Liposomes	1.44 ± 0.49†	1.52 ± 0.31†	0.21 ± 0.09
	Solution	0.15 ± 0.05	0.11 ± 0.04	—
Iris	Liposomes	0.86 ± 0.25†	1.49 ± 0.30†	0.20 ± 0.15
	Solution	0.28 ± 0.08	0.24 ± 0.041	0.19 ± 0.06
Lens	Liposomes	0.43 ± 0.11	0.27† ± 0.10	0.038 ± 0.006
	Solution	0.38 ± 0.13	0.09 ± 0.033	0.034 ± 0.011
Vitreous	Liposomes	0.084 ± 0.037	0.12 ± 0.19†	0.051 ± 0.015
	Solution	0.037 ± 0.012	0.064 ± 0.023	0.021 ± 0.008

*GCV indicates ganciclovir.

†*P* < .05 when compared with the solution group.

REFERENCES

1. Mar E, Cheng Y, Huang Y. Effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine on human cytomegalovirus replication in vitro. *Antimicrob Agents Chemother.* 1983;24:518-521.
2. Martin J, Dvorack C, Smee D, Matthews T, Verheyden J. 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine: a new potent and selective antiherpes agent. *J Med Chem.* 1983;26:759-761.
3. Cantrill H, Henry K, Melroe N, et al. Treatment of cytomegalovirus retinitis with intravitreal ganciclovir: long-term results. *Ophthalmology.* 1989;96:367-374.
4. Markham A, Faulde D. Ganciclovir: an update of its therapeutic use in cytomegalovirus infection. *Drugs.* 1994;48:455-460.
5. Maudgal P, De Clercq K, Descamps J, Missotten L. Topical treatment of experimental herpes simplex keratouveitis with 2'-O-glycylacyclovir, a water-soluble ester of acyclovir. *Arch Ophthalmol.* 1984;102:140-142.
6. Hughes P, Mitra A. Effect of acylation on the ocular disposition of acyclovir. II. Corneal permeability and anti-HSV 1 activity of 29-esters in rabbit epithelial keratitis. *J Ocul Pharmacol.* 1993;9:299-309.
7. Drew W. Is combination antiviral therapy for CMV superior to monotherapy? *J Clin Virol.* 2006;35:485-488.
8. Wiltink E, Stekkinger P, Brakenhoff J, Danner S. Determination of 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) in biological fluids by reverse-phase high pressure liquid chromatography. *Pharm Weekbl Sci.* 1987;9:261-264.
9. Choonara Y, Pillay V, Carmichael T, et al. An in vitro study of the design and development of a novel doughnut-shaped minitablet for intraocular implantation. *Int J Pharm.* 2006;310:15-24.
10. Law S, Huang K, Chiang C. Acyclovir-containing liposomes for potential ocular delivery: corneal penetration and absorption. *J Control Release.* 2000;63:135-140.
11. Institute of Laboratory Animal Resources. *Guide for the Care and Use of Laboratory Animals.* Washington, DC: National Academy Press; 1996.
12. Szoka F, Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. *Proc Natl Acad Sci USA.* 1978;75:4194-4198.
13. Wu P. The characterization and biodistribution of cefoxitin-loaded liposomes. *Int J Pharm.* 2004;271:31-39.
14. Law S, Hung H. Properties of acyclovir-containing liposomes for potential ocular delivery. *Int J Pharm.* 1998;161:253-259.
15. Durrani A, Farr S, Kellaway I. Influence of molecular weight and formulation pH on the precorneal clearance rate of hyaluronic acid in the rabbit eye. *Int J Pharm.* 1995;118:243-250.
16. McSharry J, McDonough A, Olson B, et al. Inhibition of ganciclovir-susceptible and -resistant human cytomegalovirus clinical isolates by the benzimidazole L-riboside 1263W94. *Clin Diagn Lab Immunol.* 2001;8:1279-1281.
17. McCalden T, Levy M. Retention of topical liposomal formulation on the cornea. *Experientia.* 1990;46:713-715.
18. Pleyer U, Lutz S, Jusko W, et al. Ocular absorption of topically applied FK506 from liposomal and oil formulations in the rabbit eye. *Invest Ophthalmol Vis Sci.* 1993;34:2737-2742.
19. Elsayed M, Abdallah O, Naggar V, et al. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. *Int J Pharm.* 2006;322:60-66.