Effect of Mono- and Di-Acylation on the Ocular Disposition of Ganciclovir: Physicochemical Properties, Ocular Bioreversion, and Antiviral Activity of Short Chain Ester Prodrugs

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ABSTRACT: A series of short-chain carboxylic mono- and diesters of ganciclovir were synthesized in our laboratory. Physico-chemical properties, i.e., solubility (pH 4.2), partition coefficient in 1-octanol/phosphate buffer (pH 7.4), aqueous stability at various pH values, bioreversion kinetics in various ocular homogenates and effectiveness against various Herpes viruses *in vitro* were determined. The compounds exhibited a decrease in solubility as the ester length ascended with a corresponding increase in the octanol/buffer partition coefficient values. All of the prodrugs exhibit stability profiles typical of a carboxylic ester with maximum stability at neutral or slight acidic pH (4.0–7.0). Apparent first-order rate constants associated with prodrug to drug hydrolysis in the ocular homogenates varied depending on the size of the promoiety, lipophilicity of the compound, and the ocular tissue studied. The acetyl and butyryl mono and diesters were screened against various Herpes viruses. The monobutyrate ester of ganciclovir exhibits excellent activity against HSV-2 and VZV and provides a very high selectivity index against most of the viruses studied. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:660–668, 2002

Keywords: mono- and di-acylation; ocular disposition; ganciclovir

INTRODUCTION

Human Cytomegalovirus (CMV) retinitis is the most common ocular opportunistic infection occurring in HIV-infected patients. It occurs in about 20–40% of AIDS patients, especially in individuals with blood CD4 cell counts less than 50/μL.[1] Focal yellowish-white granular patches, large areas of retinal hemorrhage with necrosis, diffuse edema are symptoms of advanced CMV retinitis, and if left untreated can lead to blindness. Many agents are available to treat this infection, including intravenous ganciclovir, foscarnet and cidofovir, slow release ganciclovir implants, and intravitreal injections of ganciclovir or foscarnet. Ganciclovir (GCV), an acycloguanosine analog, was the first drug to show efficacy in HIV-associated CMV retinitis, and hence, is still the most commonly used anti-CMV drug for treatment.[2] GCV was primarily administered systemically, but high systemic doses (5–10 mg/kg) lead to severe side effects like neutropenia, thrombocytopenia, and abnormal liver function. GCV, being a hydrophilic molecule, is occluded out by the Blood–Retinal Barrier (BRB), which prevents the entry of GCV into the eye after systemic administration. Oral delivery of GCV produces very low bioavailability (6%), and hence, is not appropriate for the treatment in the induction phase of treatment, which requires high levels of GCV in the eye. Hence, local intravitreal injections (0.2–0.4 mg), which minimize systemic

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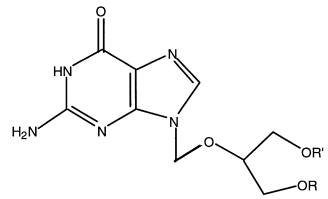
toxicities, have become the route of choice as the drug is delivered directly to the target area. But this mode of delivery has its own limitations. GCV is rapidly eliminated from the vitreous (elimination $t_{1/2}$ is approx.13 h in humans[3]), and hence, repeated intravitreal injections are necessary leading to side effects like endophthalmitis, retinal detachment, vitreal hemorrhage, and optic atrophy in the long run. Many delivery systems like intraocular implants, liposomes, and microspheres have been developed to reduce repeated administration of GCV with varying degrees of success. Intraocular implants now serve as a mode for maintenance therapy, with intravitreal injections still being the route of choice for the induction phase of treatment.[2]

Lipophilic chemical modification has been widely used for various hydrophilic drugs to improve their ocular bioavailability.[4–8] The lipophilic prodrug modification may release GCV continuously in the eye, depending on the rate of hydrolysis and elimination of the pro moiety. Also, if lipophilic prodrugs of GCV are delivered directly into the eye, the levels of GCV may be maintained for a prolonged period of time compared to GCV. Prolonged intravitreal GCV levels may be attributed to two factors, i.e., the slow hydrolysis of the prodrug to regenerate GCV and melanin binding to ocular tissues such as the retina and iris ciliary body. Unpublished *in vitro* studies in our laboratory have shown that as the lipophilicity of the pro moiety is ascended, binding to natural melanin progressively increases. To this end, lipophilic prodrug moieties ranging from the monoacetate to the monovalerate and from the diacetate to di-isobutyrate have been synthesized in our laboratory (Scheme 1). This research article focuses on these short-chain aliphatic mono- and di-ester prodrugs of GCV. Physico-chemical characterization, ocular tissue hydrolysis, and antiviral screening have been carried out to determine the optimal prodrug or prodrugs from the eight ester prodrugs studied. The objective of this research is to optimize the physico-chemical properties and enzymatic hydrolysis of GCV prodrugs for increased intraocular bioavailability.

MATERIALS AND METHODS

GCV was obtained as a gift from Hoffman La Roche (Nutley, NJ). All the prodrugs were synthesized in our laboratory according to published procedures.[9] All reagents used were of HPLC grade. New Zealand albino male rabbits weighing between 2–2.5 kg were obtained from Myrtle's Rabbitry, Thompson Station, TN.



Compound		R	R'
Monoacetate	(GCVMA)	H	$COCH_3$
Monopropionate	(GCVMP)	H	$COCH_2CH_3$
Monobutyrate	(GCVMB)	H	$CO(CH_2)_2CH_3$
Monovalerate	(GCVMV)	H	$CO(CH_2)_3CH_3$
Diacetate	(GCVDA)	$COCH_3$	$COCH_3$
Dipropionate	(GCVDP)	$COCHCH_3$	$COCHCH_3$
Dibutyrate	(GCVDB)	$CO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$
Diisobutyrate	(GCVDIB)	$COCH_2(CH_3)_2$	$CO\,CH_2(CH_3)_2$

Scheme 1. Structures of various GCV ester prodrugs synthesized.

Physicochemical Properties

Stability Studies

Six pH values, 1.2, 2.6, 4.2, 5.6, 7.4, and 9.0 were selected. HCl (pH 1.2 and 2.6), phthalate (pH 4.2 and 5.6), phosphate (pH 7.4), and boric acid (pH 9.0) buffers were prepared and ionic strength was adjusted at 0.1 M. The electrode was calibrated at 25°C, and pH of the buffers was measured before each study. Methanolic stock solutions of the prodrugs (1–3 mg/mL) were prepared and used immediately for stability studies. Aliquots (9.8 mL) of the buffer were placed in a screw capped vial and allowed to equilibrate at 37°C. Prodrug stock solution (0.2 mL) was subsequently added to the buffer, thus adjusting the total methanol concentration to 2% (v/v). Controls containing methanol and the prodrug were also carried out simultaneously to determine any degradation due to methanol. The vials were placed in a constant shaker bath set at 37°C and 60 rpm. Samples (0.2 mL) were collected at appropriate time intervals for up to 24 h. The samples were immediately stored at −80°C. During analysis the samples (one by one) were thawed and immediately analyzed. All experiments were conducted at least in triplicate.

Solubility Studies

Solubility determinations were carried out at pH 4.2, where the prodrugs exhibited maximal

stability. To establish equilibration time, a time-dependent study was carried out in the case of GCV monoacetate (GCVMA). For the rest of the prodrugs excess amount of the prodrug was placed in 3-mL buffer in a screw-capped vial. The vials were placed in a constant temperature shaker bath at 37°C and 60 rpm for 24 h. At the end of this time period, the samples were filtered through 0.45-μM Nalgene syringe filter membranes. The initial 1 mL of the filtrate was discarded. All experiments were conducted in triplicate.

Partition Coefficient Determination

Partitioning studies were performed with *n*-octanol and pH 7.4 Isotonic Phosphate Buffer (IPBS). The two phases were presaturated with each other for 48 h. After presaturation, 5 mL aliquots of octanol-saturated IPBS were placed in screw-capped vials and 0.2-mL stock solution of the prodrug prepared in IPBS was added. The mixture was vortexed and an initial sample (0.2 mL) was collected to determine the initial aqueous phase concentration C_{aqi}. Subsequently, 5 mL of IPBS-saturated octanol was added. The vials were then placed in a constant-temperature shaker bath (60 rpm) at 37°C for 24 h. After 24 h, the 0.2-mL sample was collected and analyzed to determine the final aqueous phase concentration C_{aqf}. Because these studies were carried out at pH 7.4 loss of drug from the aqueous phase could also be in part due to chemical degradation. Hence, controls containing the prodrug solution in IPBS were carried out. The final aqueous concentration was corrected for any degradative losses to generate C_{aqf}^*, which is the concentration in the aqueous phase after partitioning. The Partition Coefficient was determined by the ratio of C_{oct}/C_{aq} given by eq. 1:

$$(C_{aqi} - C^*_{aqf})/C^*_{aqf} \quad (1)$$

All samples obtained from the above physicochemical studies were analyzed by HPLC assay as described in the Analytical section.

Ocular Tissue Hydrolysis Studies

Preparation of Ocular Tissues

New Zealand albino male rabbits were used for this study. Animals were euthanized by a lethal injection of sodium pentobarbital to the marginal ear vein. Each eye was immediately enucleated, and the ocular surface was rinsed with physiological saline and blotted dry. About 150 mL of aqueous humor was aspirated using a 27-gauge needle. An incision was made at the back of the eye, and the vitreous humor was withdrawn with a tuberculin syringe. The cornea, lens, and iris cilary body were sequentially removed after cutting along the scleral–limbus junction. Finally, the retina–choroid layer was removed and all tissues were stored at −80°C prior to use. The tissues were homogenized in 5-mL chilled (4°C) IPBS for about 4 min with a tissue homogenizer in an ice bath. The homogenate was centrifuged at 12,500 rpm for 25 min at 4°C to remove debris, and the supernatant obtained was used for hydrolysis studies. Protein content of each supernatant was determined by using a BioRad assay with bovine serum albumin as the standard.

Hydrolysis Procedure

The supernatant was equilibrated at 37°C for about 30 min prior to an experiment. The study was initiated by the addition of 0.2 mL of an (0.1 mM) IPBS solution of a given prodrug to 0.8 mL of the supernatant. The control consisted of 0.8 mL of IPBS instead of the supernatant. Samples (50 μL) were withdrawn at appropriate time intervals for up to 24 h. The samples were immediately diluted with 50-μL chilled methanol to terminate the reaction and stored at −80°C until further analysis. The samples were thawed and centrifuged at 10,000 rpm for 10 min prior to analysis by HPLC for the intact ester prodrug and the regenerated parent drug GCV. Apparent first-order rate constants were calculated and corrected for any chemical hydrolysis observed with the control.

Antiviral Testing

The *in vitro* potency of selected prodrugs namely GCVMA, GCVMB, GCVDA, and GCVDB were determined against various Herpes viruses. The compounds were screened against HSV-1, HSV-2, EBV, VZV, and HCMV. Low-passage human fibroblast foreskin cells (HFF) were used at a concentration of 2.5×10^6 cells per mL in 0.1 mL of minimum essential medium supplemented with 10% fetal bovine serum were used. For HSV-1 and HSV-2, 1000 plaque-forming units (PFU) per well were used. CMV, VZV, and EBV were used at a concentration of 2500 PFU per well. All studies were conducted at NIAID by Dr. Christopher Tseng under the NIAID antiviral evaluation program.

Analytical Procedures

All samples obtained from the various studies were assayed using HPLC. The system comprised of a Waters 600 gradient pump, HP 1100 series fluorescence detector, and Waters 2487 UV detector.

HPLC Assay I

This method was used to analyze samples obtained from the different physico-chemical studies. The system consisted of a single C18 Luna column 4.6×250 mm (Phenomenex), and the mobile phase was composed of a mixture of 0.01 M Ammonium phosphate buffer (pH 2.5) and an organic modifier, which consisted of a 1:1 mixture of methanol and acetonitrile. The percentage of organic phase was varied to elute the different prodrugs. The samples were detected using fluorescence at ex $\lambda = 285$ nm and em $\lambda = 370$ nm (Table 1). This method provided rapid and reproducible results.

HPLC Assay II

For tissue hydrolysis studies it is imperative that a method be developed, which would quantify the diester, monoester, and the parent moiety GCV simultaneously. The method should be capable of separating the analyte peaks from any homogenate peaks. To resolve this problem a dual column method consisting of a cation exchange SCX column 4.6×250 mm from Alltech Associates connected in series with a Luna C18 column (Phenomenex) 4.6×250 mm was employed. The mobile phase was similar in composition to the first method. For the diesters, a step gradient had to be established after the monoester and GCV eluted, as the diesters were very lipophilic compared to the monoester and the GCV. The details of the method and the retention times have been summarized in Table 1. Samples were analyzed by either fluorescence or UV ($\lambda = 254$ nm) detection. It should be noted that the mono-isobutyrate prodrug synthesis was not successful, and hence, there was no standard available for identifying this compound with the HPLC assay.

RESULTS AND DISCUSSION

Physico-Chemical Properties

Aqueous Stability

The stability of the eight prodrugs was examined within the pH range pH 1.2 to pH 9.0. The effect of pH on the stability of these prodrugs was examined. Figure 1 exhibits the GCVMP stability profile. The observed profile exhibits a typical V-shape characterization of a carboxylic ester with maximal stability occurring between pH 4.0 and 5.0. All of the prodrugs studied showed similar behavior in the pH range studied with varying rate constants of degradation for specific acid and base catalyzed hydrolysis. The prodrugs followed apparent first-order degradation kinetics at all pH values (Figure 2). Lipophilicity of the prodrug does not appear to correlate with the stability of the prodrug. The esters exhibit maximum stability between pH 4 and 5.0 with moderate stability at neutral pH. Considering this fact the solubility studies were carried out at pH 4.2.

Table 1. Structures of Various GCV Ester Prodrugs Synthesized

	HPLC Assay I (Single C18 Column)		HPLC Assay II (SCX Column in Series With a C18 Column)				
Prodrug	Aq:Org	Retention Time	Mobile Phase A (Aq:Org) (First 15 min)	Mobile Phase B (ACN:MeOH) After 15 min	GCV	GCV-Monoester	GCV-Diester
GCVMA	87:13	5.2	87:13	—	11.2	13.3	—
GCVMP	80:20	4.9	80:20	—	8.9	10.9	—
GCVMB	70:30	5.1	70:30	—	8.5	12.4	—
GCVMV	70:30	8.1	70:30	—	7.7	10.7	—
GCVDA	75:25	7.9	87:13	0–35% (15–35 min)	11.2	13.3	25.1
GCVDP	65:35	9.2	80:20	”	8.9	10.9	27.3
GCVDB	55:45	11.9	70:30	”	8.5	12.4	36.9
GCVDIB	55:45	12.7	70:30	”	8.5	—	39.1

GCVMP has solution half lives of approximately 43 days, 9 days, 5 days, and half a day at pH values 4.2, 5.6, 7.4, and 1.2, respectively. The results indicate that the prodrug does not undergo significant degradation during a short stability study. But, to account for any chemical hydrolysis control studies were also carried out.

The profile in Figure 1 shows a typical V-shape profile. The smooth line fitted to the data shows a curvature close to the pka of the free amino group ($pk_a = 9.2$) on the purine ring indicating faster hydrolysis of the protonated form compared to the unprotonated species. This observation is consistent with a previously published report on Acyclovir ester prodrugs.[4] Specific acid and base catalysis as well as water-catalyzed spontaneous hydrolysis were also evident.

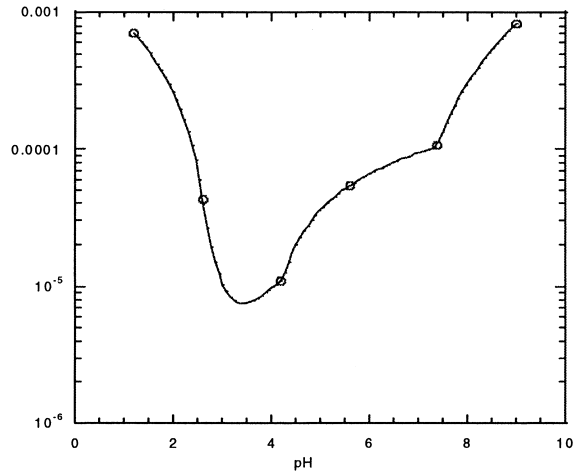


Figure 1. pH stability profile for GCVMP.

Solubility and Partition Coefficient

Aqueoous solubility of these prodrugs was determined at pH 4.2 (phthalate buffer) because the prodrugs exhibited maximal stability at this pH. A solubility time study was conducted for GCVMA to establish equilibration period, which was determined to be 24 h. All solubility determinations were carried out after 24-h equilibration. The determined values for solubility and partition coefficient have been provided in Table 2. As the number of carbons in the promoiety is gradually ascended both in the mono- and di-substituted prodrugs, lipophilicity gradually increases and solubility decreases. The solubility decrease is about fivefold for the monovalerate ester compared to the monoacetate ester, and about sixty times with the di-isobutyrate ester. Although the solubility decreases, the lipophilicity is enhanced to a larger extent with increasing carbon bulk in the ester moiety. Partition coefficient values increased by about 80-fold upon di-substitution with the butyrate pro-moiety. The results indicate that branching of the ester moiety enhances its partition into the octanol phase because of increased surface area of the carbon chain. Partition studies done previously in our laboratory have determined the log P value for GCV to be -1.55.[10] Thus, by esterification the lipophilicity of GCV has been enhanced to approximately 100 times with the monovalerate ester and upto 425 times with the dibutyrate ester. The rate-limiting barrier to transport of hydrophilic drugs is the lipophilic bilayer of the cell membrane. It may be hypothesized that the enhanced lipophilicity of

Table 2. Physio-Chemical Properties for the Ganciclovir Ester Prodrugs

Prodrug	Solubility[a]	Log P[b]
GCVMA	15.15 (± 2.55)	−1.08 (± 0.24)
GCVMP	11.89 (± 0.73)	−0.92 (± 0.05)
GCVMB	8.35 (± 0.23)	−0.30 (± 0.07)
GCVMV	4.11 (± 0.48)	−0.07 (± 0.01)
GCVDA	5.3 (± 0.25)	−0.78 (± 0.03)
GCVDP	3.26 (± 0.19)	−0.23 (± 0.07)
GCVDB	0.61 (± 0.09)	0.59 (± 0.03)
GCVDIB	0.26 (± 0.06)	0.83 (± 0.09)

Values are mean (± SD).
[a]Solubility determination carried out in pH 4.2 phthalate buffer at 37°C.
[b]Partition coefficient values (P) were carried out in 1-octanol/phosphate buffer(7.4) at 37°C.

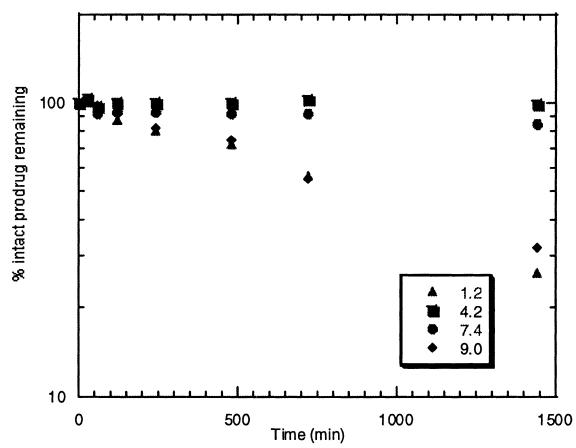


Figure 2. First-order chemical hydrolysis of GCVMV at pH 1.2, 4.2, 7.4, and 9.0.

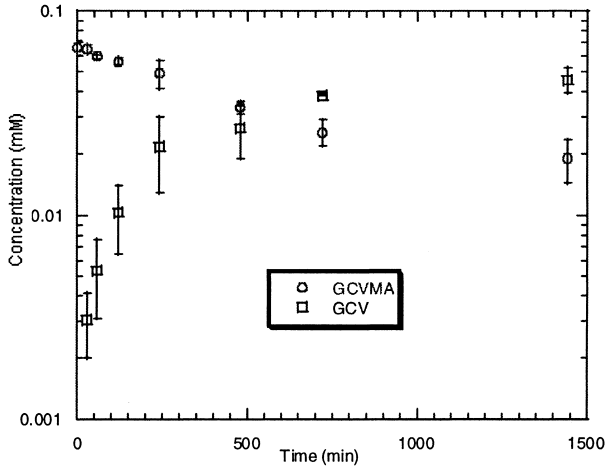


Figure 3. Regeneration of GCV from GCVMA upon hydrolysis in isolated rabbit vitreous humor.

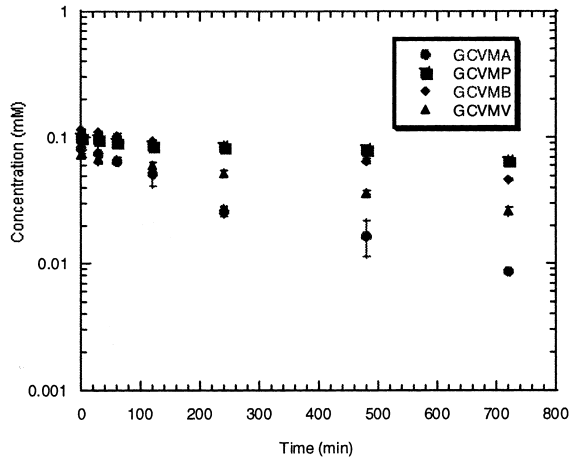


Figure 4. Hydrolysis of GCV monoesters in rabbit lens homogenate at 37°C.

the synthesized prodrugs may increase ocular bioavailability on systemic administration. The blood retinal barrier (BRB) like the blood–brain barrier prevents the entry of relatively hydrophilic molecules. Enhanced lipophilicity will help overcome this barrier, and may increase the ocular availability of GCV when these prodrugs are delivered systemically. In addition, the lipophilicity of the prodrugs may lead to enhanced melanin binding due to increased hydrophobic interactions between the prodrug and melanin, leading to formation of a reservoir depot in the retinochoroidal and iris–ciliary body.

Ocular Homogenate Studies

Apparent first-order hydrolytic rate constants associated with GCV mono- and di-esters in aqueous humor, vitreous humor, lens, iris–ciliary, cornea and retina–choroid have been presented in Table 3. The values in the table have been obtained after correcting for any chemical degradation due to IPBS. Loss of the prodrug was accompanied by regeneration of GCV in the case of monoesters (Figure 3), whereas the diester first hydrolyzes to generate the monoester, which is sequentially hydrolyzed to GCV. The combined molar amounts of the prodrug and regenerated parent compound remained constant throughout the experiment. The formation of no other metabolites like guanine indicates only esterase activity in the tissues. Ocular tissue hydrolysis shows dependence on ester chain length (Figure 4), and this finding is consistent with the chain-length dependence of ocular ester prodrug hydrolysis reported previously.[11] The results indicate that increased lipophilicity of the substrate leads to an increased interaction with the enzyme-active site. It has been hypothesized that the enzyme-active site is a lipophilic pocket.[4] As the chain length is ascended from the acetate to the valerate the rate of hydrolysis increases in most tissues. Also, the diesters cleave at a faster rate compared to the monoesters, indicating that the increased bulk at the two esterification sites did not hinder enzyme activity. However, values for the di-isobutyrate ester are less compared to the dibutyrate ester, indicating that branching of the side chain slows down the enzymatic cleavage by the various ocular tissues. This may be attributed to the increased bulk of the side chains leading to steric hindrance. The esterase activity may decrease with further increase in carbon chain because of the bulk of the pro moiety.[11] Majority of the esterase activity exhibited in ocular tissues is due to the presence of acetylcholine esterase and butyrylcholine esterases. The esterase activity of a tissue is a function of the activity exhibited by both enzymes. The proportion of each enzyme present plays an important role, as these enzymes are specific, and will have different affinities for the different pro-moieties. Generally, in most of the ocular tissues it has been shown that butyrylcholine esterases form the major portion of the esterases present in ocular tissues.[12] This could also be one important factor leading to increased hydrolysis of pro-moieties with a carbon chain length greater or equal to butyrate. Values in Table 3 indicate that the retina–choroid and iris–ciliary body show

Table 3. First-Order Rate Constants for the Hydrolysis of the GCV Ester in Ocular Tissue Homogenates at 37°C

Prodrug	Control	Vitreous Body	Aqueous Humor	Lens	Cornea	Iris Ciliary Body	Retina-Choroid
GCVMA	0.09 (±0.01)	1.74 (±0.38)	10.56 (±1.38)	3.75 (±0.49)	4.14 (±0.18)	14.88 (±3.71)	8.37 (±1.23)
GCVMP	0.11 (±0.01)	0.87 (±0.10)	5.60 (±0.44)	0.64 (±0.06)	7.60 (±0.70)	13.69 (±2.72)	14.67 (±0.69)
GCVMB	0.19 (±0.02)	2.31 (±0.14)	24.62 (±3.28)	1.43 (±0.12)	9.77 (±1.19)	87.5 (±10.71)	61.42 (±5.97)
GCVMV	0.56 (±0.06)	2.92 (±0.12)	10.69 (±1.16)	1.83 (±0.06)	43.2 (±5.79)	278.57 (±18.89)	215.9 (±14.17)
GCVDA	1.35 (±0.78)	33.34 (±1.1)	118.21 (±7.2)	175.88 (±9.56)	57.91 (±6.66)	230.17 (±11.06)	124.51 (±3.36)
GCVDP	1.14 (±0.69)	18.06 (±0.79)	39.33 (±1.4)	132.35 (±6.91)	377.83 (±21.36)	370.77 (±15.9)	364.89 (±41.4)
GCVDB	2.92 (±0.52)	33.48 (±1.67)	131.11 (±6.34)	372.15 (±14.36)	692.10 (±43.21)	3164.21 (±78.92)	3164.23 (±81.24)
GCVDIB	2.14 (±0.74)	27.01 (±2.36)	117.11 (±10.02)	296.14 (±26.78)	565.12 (±36.95)	1692.91 (±66.67)	1725.49 (±71.69)

Values are $= K \times 10^4$ min^{-1} mg^{-1} protein. (k is the pseudofirst-order hydrolysis rate).
Values are mean (±SD).

highest esterase activity compared to other ocular tissues studied. This result is expected, as blood capillaries supply the retina and the iris–ciliary body and the amount of esterase enzymes present in these tissues is higher than in avascular tissues. Vitreous humor, which will be the biological environment once the drug is administered intravitreally, also cleaves the prodrug moiety, albeit to a much lesser extent compared to other tissues.

Antiviral Testing

The acetyl and butyryl prodrugs of GCV were screened against various HSV strains. The results have been summarized in Table 4. The stability of the prodrugs was not measured in the media used for carrying out the antiviral activity determinations. These prodrugs are susceptible to chemical hydrolysis, and will definitely hydrolyze to give GCV to a certain extent. Also, the cellular enzymes present will hydrolyze the prodrugs to generate the parent drug GCV, which will elicit its antiviral activity. The activity noted for the monoester prodrugs is indeed the activity exerted by the regenerated GCV. GCV monobutyrate has similar activity as ACV and GCV against most of the strains studied. The monobutyrate has the most activity and the best selectivity index (CC_{50}/EC_{50}) compared to the other esters studied. The diester prodrugs of GCV had very little activity compared to the monoesters. A plausible explanation for this could be the fact that for antiviral activity a free hydroxyl group is required.[13,14] The diester prodrugs though lipophilic lack a free hydroxyl group, and hence, intact diester would result in little or no activity. The monobutyryl prodrug shows more activity compared to the monoacetyl prodrug, owing to the fact that the cellular esterases as in the ocular tissues may have more affinity for it. In similar studies done with ACV ester prodrugs the butyrate ester showed good activity against these herpes strains.[4] GCV monobutyrate appears to be an ideal candidate as an ocular prodrug for antiviral activity. The affinity of the enzymes for this promoiety and its sufficient lipophilicity makes it a valuable prodrug for use in ocular therapeutics.

CONCLUSIONS

The ideal ocular prodrug should be the one that possesses sufficient lipophilicity to cross the BRB

Table 4. Antiviral Screening of GCV Ester Prodrugs for Various Herpes Viruses

Entity	HSV-1 μg/mL	HSV-2 μg/mL	HCMV μg/mL	MCMV μg/mL	VZV μg/mL
ACV	$EC_{50} = 0.14$	$EC_{50} = 1.8$	—	—	$EC_{50} = 0.24$
GCV	—	—	$EC_{50} = 0.16$	$EC_{50} = 1.1$	—
GCVMA	$EC_{50} = 1.5$ $CC_{50} > 100$ $SI > 66.6$	$EC_{50} = 2.7$ $CC_{50} > 100$ $SI > 37$	$EC_{50} = 1.2$ $CC_{50} > 100$ $SI > 83.3$	$EC_{50} = 3.4$ $CC_{50} > 70$ $SI > 20.6$	$EC_{50} = 0.6$ $CC_{50} > 100$ $SI > 169$
GCVMB	$EC_{50} = 0.5$ $CC_{50} > 100$ $SI > 200$	$EC_{50} = 1.7$ $CC_{50} > 100$ $SI > 58.8$	$EC_{50} = 0.53$ $CC_{50} > 100$ $SI > 189$	$EC_{50} = 7.7$ $CC_{50} > 100$ $SI > 13$	$EC_{50} = 0.2$ $CC_{50} > 100$ $SI > 370$
GCVDA	$EC_{50} = 2.8$ $CC_{50} > 100$ $SI > 35.7$	$EC_{50} = 15.8$ $CC_{50} > 100$ $SI > 6.3$	$EC_{50} = 0.54$ $CC_{50} > 100$ $SI > 185$	$EC_{50} = 9.8$ $CC_{50} > 100$ $SI > 10.2$	$EC_{50} = 0.6$ $CC_{50} > 100$ $SI > 164$
GCVDB	$EC_{50} = 2.4$ $CC_{50} > 100$ $SI > 41.6$	$EC_{50} = 100$ $CC_{50} > 100$ $SI > 0$	$EC_{50} = 1.3$ $CC_{50} > 100$ $SI > 77$	$EC_{50} = 4.4$ $CC_{50} > 100$ $SI > 23$	$EC_{50} = 1.1$ $CC_{50} > 100$ $SI > 91$

CC_{50} (cytotoxic concentration 50) = concentration required to inhibit 50% stationary cells to take up neutral red.
EC_{50} (effective concentration 50) = concentration required to inhibit cell growth by 50%. SI (selective index) = CC_{50}/EC_{50}.

and possess sufficient solution stability for it to be formulated into an appropriate ophthalmic dosage form. The ester prodrugs must exhibit the desired hydrolytic kinetics when delivered into the eye. The prodrug must hydrolyze to generate the therapeutically active parent compound at the target site. From the various studies performed on the prodrugs synthesized so far it can be concluded that these compounds show the desired physico-chemical properties, namely enhanced lipophilicity and sufficient solution stability, between pH 4.0 and 7.0. Ocular tissue hydrolysis indicates that these prodrugs are bioconverted to the parent compound GCV, which is the therapeutically active drug. The prodrugs particularly monoesters have also shown the ability to inhibit viral growth *in vitro*, which adds to their utility in the antiviral therapeutics.

In summary, the prodrugs investigated thus far show positive contributions towards the development of an ideal prodrug for enhancing the ocular bioavailability of GCV. It would be interesting to see how longer chained esters and charged esters of GCV perform under similar experimental conditions. The longer chained esters may release the parent moiety very slowly in the eye owing to their size. This may prove to be useful for long-term therapeutics. Melanin binding capacity of these esters and the esters studied here needs to be investigated. All these studies will be the topic of investigation in our future article with these series of compounds.

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