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Enhanced Delivery of Ganciclovir to the Brain through the Use of Redox Targeting

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Enhanced delivery of ganciclovir to the brain was demonstrated by a redox-based chemical delivery system. A ganciclovir monoester in which a 1-methyl-1,4-dihydronicotinate was covalently attached to one of the hydroxymethyl functions was prepared. The stability of the ganciclovir chemical delivery system (DHPG-CDS) was evaluated in aqueous buffers and organ homogenates. In vivo distribution studies in the rat indicated that while ganciclovir poorly penetrated into the central nervous system and was rapidly eliminated, DHPG-CDS provided for therapeutically relevant (2.7 μM) and sustained levels of the parent compound through 6 h. An analysis of the area under the concentration curve indicated that the chemical delivery system delivered five times more ganciclovir than that of the parent drug. The high levels in the brain and reduced levels in the blood gave a brain-to-blood drug concentration ratio of 2.54 for ganciclovir when delivered by the chemical delivery system, compared to a ratio of 0.063 when the parent drug was administered. These data suggest that DHPG-CDS could be a useful adjunct for the treatment of cytomegalovirus encephalitis.

Cytomegalovirus (CMV) is a herpesvirus which, in common with other members of this group, leads to a latent disease state during which the viral genome becomes incorporated into the host DNA (19, 21). While CMV infections are common, they are usually benign. There are, however, several circumstances in which CMV infection can product high morbidity and mortality, including in infants and in immunocompromised individuals. The largest upsurge in human CMV infection has been related to the modern plague of AIDS, in which immunosuppression is a hallmark. Human CMV occurs in 94% of all patients suffering with AIDS and has been implicated as a deadly cofactor (11, 33). The virus is thought to be responsible for several clinical presentations, including retinitis (31), pneumonitis (28), and encephalitis (29, 35). Neural involvement of human CMV is widespread but can be proven only by biopsy or postmortem examination. The defining histological evidence for CMV infection in the brain is microglial nodules (18). While central transmission is common, the neurotropic aspects of the causative AIDS pathogen, human immunodeficiency virus type 1, often complicate a clear interpretation of the central cytomegalic disease. In general CMV is thought to produce a subacute encephalopathy similar to that seen with human immunodeficiency virus.

A significant advance in the treatment of human CMV infection came with the discovery and development of ganciclovir, a hydroxymethyl analog of acyclovir (2, 26, 30). Ganciclovir inhibits human CMV reproduction through the action of its triphosphate anabolite which prevents viral DNA replication at the level of DNA polymerase. Administration of ganciclovir to infected patients has demonstrated dramatic improvements in retinitis, with significant reduction in viral titers and clinically important improvements in sight (16, 31). Studies on the treatment of encephalitis have not been encouraging (13, 22). The poor performance of ganciclovir against encephalitis is no doubt related to the low lipophilicity of the drug and its inability to efficiently penetrate various biological membranes, including the blood-brain barrier.

The foregoing discussion suggests that improved therapy for human encephalitic CMV infection might be obtained by increasing the concentration of the drug at its site of action, i.e., the central nervous system (CNS). Such improvement might be achieved through the use of a brain-targeting chemical delivery system (CDS) (5, 6, 8). This technology involves the covalent attachment of a redox targetor to the compound of interest, which provides for an increase in brain uptake due to enhanced lipophilicity. Unlike simple prodrugs, however, the targetor is designed to undergo an enzymatically mediated oxidation that converts the membrane-permeating transport system into a hydrophilic, membrane-nonpermeating conjugate. This polar conjugate is readily eliminated from the systemic circulation but is somewhat retained behind the blood-brain barrier, generating a favorable brain-versus-blood drug concentration ratio as a function of time. The "lock-in" conjugate can then hydrolyze, releasing the parent drug with some selectivity in the CNS. While a variety of targetors have been examined, derivatives of the dihydronicotinate-nicotinate redox couple have proven to be the most successful. Application of the approach to a number of drugs, including antiviral nucleosides, has been reported (1, 4, 7, 9, 10, 24, 25).

MATERIALS AND METHODS

Drugs and chemicals. Microcombustion analyses of compounds prepared were performed by Atlantic Microlabs (Atlanta, Ga.). Uncorrected melting points were determined with an Electrothermal melting-point apparatus. UV spectra were recorded on either a Hewlett-Packard 8451A diode array or a Shimadzu UV-160 rapid-scan spectrophotometer. Nuclear magnetic resonance spectra (NMR) were obtained with a Varian VXR-300 (300-MHz, FT mode) spectrometer. Samples were dissolved in an appropriate deuteriated solvent, and chemical shifts (δ) were reported relative to an internal standard (tetramethylsilane). Solvents and reagents were ob-

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tained from Baxter Scientific or Aldrich Chemical Co. Ganciclovir was a gift from Syntex, Inc.

(i) 9-[(3-Hydroxy-1-*tert*-butyldimethylsilyloxy-2-propoxy) methyl]guanine (compound 2 in Fig. 1). To a solution of 4.5 g (17.63 mmol) of ganciclovir (compound 1 in Fig. 1) in 500 ml of dimethylformamide, 4 g (26.54 mmol) of *tert*-butyldimethylsilyl chloride and 3 g (44.06 mmol) of imidazole were added. The mixture was stirred at 50°C for 16 h and at 25°C for 7 days. Additional amounts of imidazole (3 × 0.75 g) and *tert*-butyldimethylsilyl chloride (3 × 1.0 g) were added during this time to complete the reaction as indicated by the disappearance of ganciclovir. The solvent was reduced in vacuo to approximately 50 ml, and the residue was diluted with water. The formed precipitate was filtered, washed with water, and dried. The crude material, which consisted of a mixture of the monosilyl (compound 2) and bissilyl (compound 2a) derivatives was subjected to open-column chromatography (150 g of silica gel [Davisil] grade 634, 100/200 mesh, 60-Å [6.0-nm] pore size). The monosilyl derivative was obtained with a mobile phase containing chloroform and methanol (85:15). This gave 3.0 g of product (yield = 48%): 245 to 247°C mp; ^{1}H NMR (dimethyl sulfoxide [DMSO]-d_6) δ 7.80 (s, 1H, C-8 proton), 6.50 (bs, 2H, NH_2), 5.40 (bs, 2H, C-1′ proton), 4.70 (m, 1H, C-3′ proton), 3.50 (m, 3H, C-4′ and C-5′ protons), 0.90 [s, 9H, $C(CH_3)_3$], 0.10 [s, 6H, $Si(CH_3)_2$].

(ii) 3-[[9-(3-Hydroxypropoxy)methyl]guanidinyl]-1-methylpyridinium iodide (DHPG-Q$^+$, compound 4 in Fig. 1). To a solution of 1.87 g (5 mmol) of compound 2 in 100 ml of dry dimethyformamide, a mixture of 2.21 g (5 mmol) of the mixed anhydride 3-(2,6-dichlorobenzoyl)carbonyl-1 methylpyridinium iodide (compound 3 in Fig. 1) and 0.61 g (5 mmol) of 4-dimethylaminopyridine was added. The mixed anhydride (compound 3) was prepared by reacting nicotinic acid with 2,6-dichlorobenzoic acid in benzene with triethylamine as a proton scavenger, and then the resulting anhydride was quaternized with methyl iodide in acetonitrile. The mixture of compounds 2 and 3 was stirred at 20 to 25°C for 2 days with exclusion of moisture and under argon. The precipitated solid (2,6-dichlorobenzoic acid) was removed by filtration. To the filtrate was then added 3.30 g (12.6 mmol) of tetrabutylammonium fluoride, and the system was stirred for 2 days at 20 to 25°C. The solvent was removed in vacuo to leave a gum that was triturated with acetone. The solid produced was filtered and used without further purification.

(iii) 9-[1-[[(1,4-Dihydro-1-methylpyrin-3-yl)carbonyl]oxy]-3-hydroxy-2-propoxymethyl]guanine (DHPG-CDS, compound 5 in Fig. 1). DHPG-Q$^+$ (2.55 g, 5 mmol) was dissolved in 100 ml of cold, degassed water. To this solution was added 2.56 g (30 mmol) of sodium bicarbonate and 3.53 g (20 mmol) of sodium dithionite. The mixture was stirred for 1 h, at which time the formed precipitate was removed by filtration, washed with cold water, and dried in vacuo, giving 0.8 g of DHPG-CDS: 198°C mp (dec.); high-performance liquid chromatography (HPLC), 98%; ^{1}H NMR (DMSO-d_6) δ 7.90 (s, 1H, C-8 proton), 6.8 (d, 1H, dihydropyridine C-2 proton), 6.50 (bs, 2H, NH_2), 5.80 (m, 1H, dihydropyridine C-6), 5.50 (bs, 2H, C-1′ proton), 4.80 (m, 1H, dihydropyridine C-5 proton), 4.00 (m, 2H, C-3′ and C-4′ protons), 3.50 (bs, 1H, OH), 3.00 (m, 5H, dihydropyridine C-4 and N-CH_3 protons). Analysis calculated for $C_{16}H_{20}N_6O_5 \cdot 3/2H_2O$: C, 47.64%; H, 5.62%; N, 20.83%. Found: C, 47.92%; H, 5.63%; N, 20.63%.

Analytical methodology. HPLC was used in the detection, separation, and quantitation of the compounds of interest. The chromatographic system consisted of a Perkin-Elmer Series 4 microprocessor-controlled solvent delivery system, a Perkin-Elmer ISS-100 autosampler, a Kratos Spectroflow 757 UV/VIS variable-wavelength detector, and a SpectraPhysics model 4290 integrator. Compounds were chromatographed on an analytical column (25 cm by 4.6 mm [inside diameter]; Spherisorb ODS-2; 5-μm particle size; Alltech, Inc.) fitted with a guard column and a 2-μm-pore-size in-line filter. All determinations were performed at ambient temperature, and analytes were detected at 254 nm. For buffer and homogenate analyses, the mobile phase used to elute DHPG-Q$^+$ and ganciclovir contained 10 mM KH_2PO_4-acetonitrile (4:1) flowing at 1.0 ml/min. Under these conditions, DHPG-Q$^+$ eluted at 5 min while ganciclovir had a retention time of 3 min. For DHPG-CDS, a mobile phase containing water-acetonitrile (80:20) flowing at 1.0 ml/min was used. For DHPG-CDS, the retention time was 5.2 min in this system. For analysis of ganciclovir and DHPG-Q$^+$ in biological tissue derived from the tissue distribution studies, a different method was applied. In this system, the mobile phase contained 95% 10 mM KH_2PO_4, 5% methanol, and 1.0 mM tetramethyl ammonium perchlorate as a competing salt. Ganciclovir and the corresponding quaternary salts had retention times of 8 and 14.5 min, respectively, in this system. Standard curves were linear over the concentration range of interest ($r > 0.999$). The limits of accurate quantitation of ganciclovir in the biological tissues examined (rat brain, lung, and blood) were 50 ng/ml or g (coefficient of variation, 5.2%) and 275 ng/ml or g for DHPG-Q$^+$ (coefficient of variation, 6.3%).

Physicochemical and stability studies. The apparent partition coefficients (PCs) for ganciclovir, DHPG-Q$^+$, and DHPG-CDS were determined in an octanol-water system (23). After equilibration of *n*-octanol in water, the drugs of interest were dissolved in the appropriate phase (DHPG-CDS in octanol and DHPG-Q$^+$ and ganciclovir in the aqueous phase). Typically, 0.5 ml of the drug solution was allowed to equilibrate with the second phase by using various volumes (5 to 15 ml). The phases were then separated by centrifugation (7,000 rpm for 2 min), and the concentration of the object compounds was determined by HPLC as described above. The stability of DHPG-CDS and the corresponding quaternary salt was determined in a number of buffer systems over a wide pH range (pH 4 to 10). All studies were completed at 37°C at constant ionic strength (μ = 0.01 M). The buffers used were acetate in the pH range of 4 to 5, phosphate in the pH range of 6 to 8, and carbonate in the pH range of 9 to 10. At pH values of <7, the rate of DHPG-CDS was monitored by UV spectrophotometry of the 360 band III absorbance. Kinetic determinations were made with the aid of a dedicated HP 85 microprocessor and a data acquisition and manipulation software program written for the computer. Kinetic experiments were performed using a 2.5-ml portion of the buffer maintained in a thermostated cell holder. A concentrated solution of the compound of interest was prepared in DMSO, and a portion (25 μl) was added to the buffer to give an initial drug concentration of 50 μM. At pH values of >7 for DHPG-CDS and for all studies involving DHPG-Q$^+$, the rate of degradation in buffer was monitored by HPLC using the method described above. In this series of experiments, a 50-μl aliquot of a DMSO solution of the compound of interest was added to 5 ml of the appropriate buffer maintained in a thermostated water bath. As in the UV experiments, the initial drug concentration was 50 μM. At various times after drug addition to the buffers, samples were withdrawn and analyzed. In both the UV and HPLC experiments, pseudo-first-order conditions were maintained. Rate constants were obtained from the slope of a plot of either log peak height or log absorbance as a function of time. In these experiments, the disappearances of DHPG-CDS and DHPG-Q$^+$ were monitored as were the appearances of

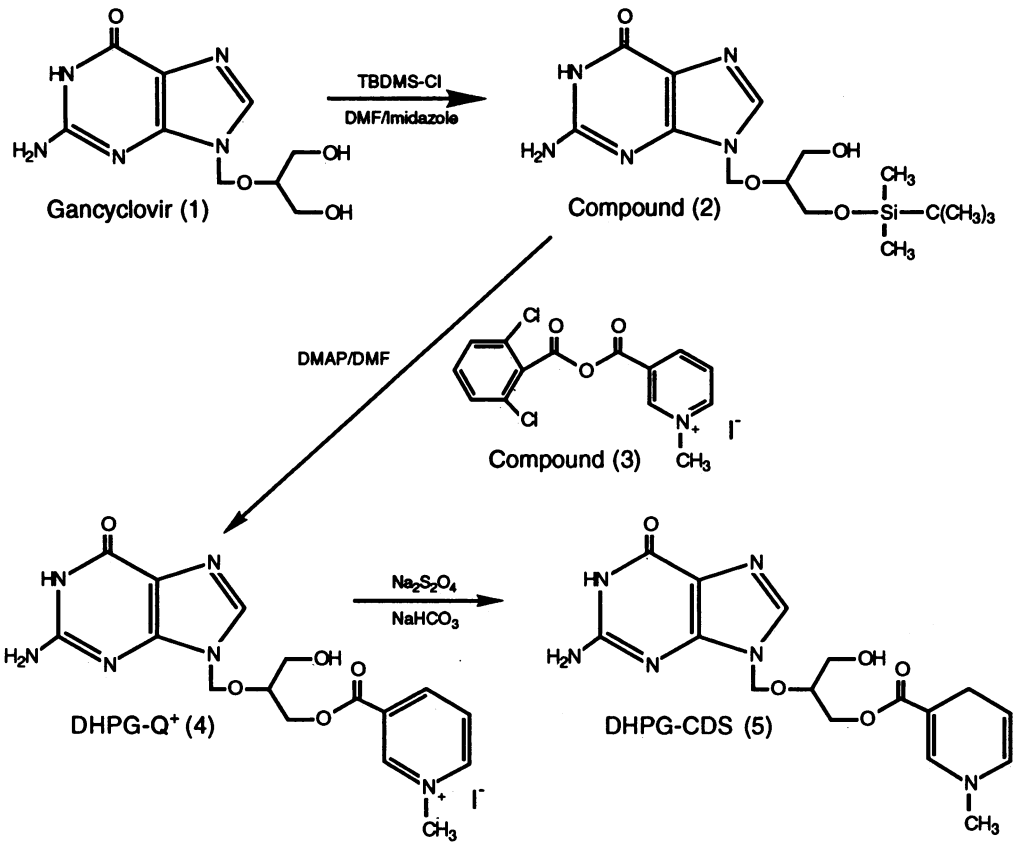

FIG. 1. Synthesis of DHPG-CDS as well as the precursor quaternary salt (DHPG-Q$^+$).

DHPG-Q$^+$ and ganciclovir. Degradation of ganciclovir to guanine was not modeled in our analysis. All experiments monitored buffer-induced degradation over at least three half-lives ($t_{1/2}$s). For in vitro homogenate studies, freshly obtained rat (Sprague-Dawley) tissues (blood and brain) or human blood was used. Brain tissue was homogenized with a Potter-Elvenjen glass tube and Teflon pestle in cold isotonic phosphate-buffered saline to give a 20% (wt/vol) homogenate. Blood or brain homogenate was then placed in a Teflon-lined screw-top vial and equilibrated at 37°C. A DMSO solution of the appropriate compound was then added to the biological matrix to produce an initial concentration of 50 μM. At various times after the addition, 200 μl of the sample was removed and added to 800 μl of ice-cold acetonitrile. The suspension was centrifuged at 10,000 rpm for 3 min. The supernatant was withdrawn, filtered through a 0.45-μm-pore-size syringe filter (Millipore, Inc.), and analyzed by HPLC as described above. Kinetics of degradation for the systems described were pseudo-first order, and rate constants (k) were determined from the negative of the slope of a natural log plot of the change in chromatographic peak height with time. $t_{1/2}$s were calculated as (ln 2)/k.

Tissue distribution in rats. Conscious Sprague-Dawley rats (body weight = 200 g) were restrained in a Broome-type holder and were injected intravenously via the tail vein with either ganciclovir or DHPG-CDS. The vehicle for both compounds was DMSO (0.5 ml/kg of body weight), and the drug dose was maintained at 80 μmol/kg (20 mg of ganciclovir per kg and 30 mg of DHPG-CDS per kg). At various times subsequent to drug administration (15 and 30 min and 1, 2, 4, 6, and 24 h), animals were sacrificed by rapid decapitation and brains, lungs, and trunk blood were collected. The blood was collected into heparinized tubes and centrifuged at 3,000 rpm for 10 min, and the plasma was separated and stored at −20°C. Organs were collected, weighed, and stored on dry ice prior to sample preparation. Plasma samples (1.0 ml) were then diluted with 4 ml of a mixture of equal volumes of acetonitrile and 0.01 M phosphate buffer (pH 3.2) and centrifuged at 12,000 rpm for 15 min, and the supernatant was separated and filtered for HPLC analysis. The organs (1 to 2 g) were homogenized in a mixture of acetonitrile and phosphate-buffered saline and centrifuged at 12,000 rpm for 15 min. The supernatant was filtered and analyzed by HPLC. Area under the curve analyses were done using the RSTRIP software package (MicroMath, Inc.)

RESULTS

Chemistry. DHPG-CDS was synthesized according to Fig. 1 in which ganciclovir was reacted with *tert*-butyldimethylsilyl chloride to give equal quantities of the monosilyl and bissilyl protected compounds. This mixture was separated by preparative open-column chromatography to give the pure monosilyl protected ganciclovir derivative. The 1-methylnicotinate ester was then attached in a single step using the novel acylating agent 1-(2,6-dichlorophenyl)-3-(1-methyl-3-pyridinyl)carbonic anhydride. The development of this reagent was necessary since methylation of a nicotinate ester would likely be complicated by side reactions, specifically guanine N^7 alkylation. During reaction workup the protecting silyl group was re-

TABLE 1. k_{obs}, $t_{1/2}$s, and correlation coefficients (r) for decomposition of DHPG-CDS and DHPG-Q$^+$ in aqueous buffers

Drug	pH	k_{obs} (min^{-1})	$t_{1/2}$ (min)	r
DHPG-CDS	4.00	0.0445	15.29	0.990
	4.51	0.0208	33.16	0.993
	5.00	0.0078	87.89	0.997
	6.00	0.0042	164.02	0.995
	6.63	0.0029	236.23	0.996
	7.04	0.0018	384.60	0.998
	7.40	0.0012	540.77	0.999
	8.15	0.0008	840.62	0.998
DHPG-Q$^+$	6.00	0.0002	2,783.70	0.985
	7.04	0.0018	374.99	0.997
	7.40	0.0053	128.59	0.989
	8.15	0.0204	33.90	0.988
	9.08	0.1371	5.05	0.999
	10.00	0.6671	1.04	0.996

moved, yielding DHPG-Q$^+$, which upon sodium dithionite reduction gave rise to DHPG-CDS.

Lipophilicity. The octanol-water PCs for ganciclovir, DHPG-Q$^+$, and for DHPG-CDS were determined by traditional shake-flask methods. The log PC for ganciclovir was found to be -1.95, consistent with previously determined values (3, 20). DHPG-CDS was found to be 55 times more lipophilic than ganciclovir (log PC = -0.20), while DHPG-Q$^+$ was more than twofold less lipophilic (log PC = -2.33).

Stability in buffers. The stability of both DHPG-Q$^+$ and DHPG-CDS was determined in a variety of buffer systems, with results reported in Table 1 and Fig. 2. The logarithms of the observed rate constants (k_{obs}) were linear as a function of pH for the quaternary salt and the dihydronicotinate. The following equations were generated: log k_{obs} = 0.116 − 0.405 pH (DHPG-CDS) and log k_{obs} = −8.93 + 0.883 pH (DHPG-Q$^+$). Since extrapolation to zero buffer concentration was not performed, the rate values include in their magnitude both specific and potentially general catalytic terms. Given this caveat, it is clear that the stability of the CDS decreases as the pH decreases while DHPG-Q$^+$ increases in stability with an increase in pH. This profile is consistent with the documented acid lability of dihydronicotinate, which typically undergoes acid-catalyzed irreversible water addition across the 5,6-double bond, resulting in the formation of a 6-hydroxytetrahydropyridine species. The acid-catalyzed degradation product is detected in the buffer samples. In the case of DHPG-Q$^+$, base-catalyzed ester hydrolysis is the likely cause for the instability at high pH values.

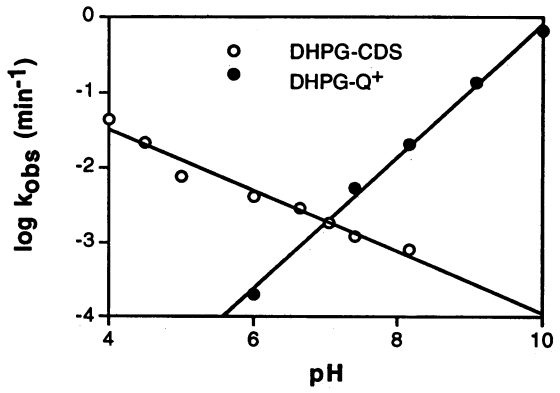

FIG. 2. pH Rate (k_{obs}) profile for DHPG-CDS and DHPG-Q$^+$ in aqueous buffer solutions.

TABLE 2. k_{obs}s, $t_{1/2}$s, and correlation coefficients (r) for decomposition of DHPG-CDS and DHPG-Q$^+$ in various biological matrices

Drug	Matrix	k_{obs} (min^{-1})	$t_{1/2}$ (min)	r
DHPG-CDS	Rat blood	0.0127	54.54	0.905
	Rat brain (20%)	0.0803	8.63	0.995
	Human blood	0.0231	29.95	0.928
DHPG-Q$^+$	Rat blood	0.0154	44.96	0.993
	Rat brain (20%)	0.0204	33.95	0.991
	Human blood	0.0132	52.30	0.982

Stability in tissue homogenates and blood. Results of the stability studies in rat brain homogenates and human and rat blood are given in Table 2. The data indicate rapid oxidation of DHPG-CDS in 20% rat brain homogenate, with a measured $t_{1/2}$ of 8.6 min. This represents a substantial acceleration of the degradation rate observed in pH 7.4 phosphate buffer ($t_{1/2}$ = 9 h) and is indicative of an enzyme-mediated oxidation. Oxidation of DHPG-CDS in whole blood was slower than that observed in brain homogenate, with pseudo-first-order $t_{1/2}$s of 54 and 30 min for rat and human blood, respectively. In these studies, no acid-catalyzed addition product was observed. DHPG-Q$^+$ was hydrolyzed with a $t_{1/2}$ of between 34 and 52 min in the three biological matrices examined, and the acceleration over the rate of hydrolysis in buffer ($t_{1/2}$ = 2 h) was not as dramatic as that seen for buffer versus enzymatic oxidation of DHPG-CDS.

Tissue distribution studies in the rat. Results of the tissue distribution study of both intravenously administered ganciclovir and DHPG-CDS are presented in Tables 3 and 4 and in Fig. 3. Administration of ganciclovir to rats produced a distributional profile consistent with the polar nature of the drug. High initial levels of drug were observed in the blood and lungs, with poor penetration into the brain. Levels in the blood fell rapidly from an initial value of 22 μg/ml at 15 min to undetectable levels at 2 h. The estimated $t_{1/2}$ of ganciclovir in blood was 30 min (r = 0.94). A similar profile was observed in the lung, with initial high drug levels disappearing by 2 h. In the brain, the concentration of ganciclovir was highest at 15 min but was only 2.5% of the initial levels in blood. The concentrations in the brain rapidly fell below the therapeutic window and were undetectable at 4 h, with an apparent $t_{1/2}$ of disappearance of 60 min (r = 0.88). After administration of DHPG-CDS a significantly different profile was obtained. DHPG-Q$^+$ was observed in high concentrations in both the lung and blood, indicative of extensive tissue distribution by DHPG-CDS (Table 5). Detectable levels of the quaternary salt which were

TABLE 3. Levels of ganciclovir in various tissues after administration of a 20-mg/kg (80-μmol/kg) dose of ganciclovir

Time (h)	Ganciclovir level[a] ± SEM in:		
	Plasma (μg/ml)	Lung (μg/g)	Brain (μg/g)
0.25	22.01 ± 2.08	21.59 ± 2.35	0.60 ± 0.05
0.50	6.59 ± 1.53	10.35 ± 0.53	0.33 ± 0.04
1	3.32 ± 0.60	6.88 ± 0.92	0.20 ± 0.02
2	—	—	0.16 ± 0.05

[a] —, greater than the limit of detection but less than the limit of quantitation. At 4, 6, and 24 h levels in all tissues were lower than the limit of detection.

TABLE 4. Levels of gancyclovir in various tissues after administration of a 30-mg/kg (80-μmol/kg) dose of DHPG-CDS

Time (h)	Ganciclovir level[a] ± SEM in:		
	Plasma (μg/ml)	Lung (μg/g)	Brain (μg/g)
0.25	1.35 ± 0.05	0.53 ± 0.03	0.69 ± 0.03
0.50	0.82 ± 0.04	0.51 ± 0.06	0.71 ± 0.01
1	0.37 ± 0.04	0.43 ± 0.05	0.63 ± 0.03
2	0.33 ± 0.02	0.32 ± 0.04	0.49 ± 0.02
4	0	0.27 ± 0.04	0.65 ± 0.04
6	0	0.23 ± 0.02	0.66 ± 0.07

[a] 0, lower than the limit of detection. At 24 h, levels in all tissues were lower than the limit of detection.

TABLE 5. Levels of DHPG-Q^+ in various tissues after administration of a 30-mg/kg (80-μmol/kg) dose of DHPG-CDS

Time (h)	DHPG-Q^+ level[a] ± SEM in:	
	Plasma (μg/ml)	Lung (μg/g)
0.25	29.48 ± 2.82	7.21 ± 0.53
0.50	17.57 ± 0.61	8.86 ± 0.74
1	15.76 ± 0.18	5.54 ± 1.53
2	15.15 ± 3.50	3.74 ± 0.38
4	8.76 ± 2.41	3.55 ± 0.69
6	5.31 ± 0.53	1.63 ± 0.81

[a] In the brain, levels from 0.25 to 6 h were greater than the limit of detection but less than the limit of quantitation; in all tissues at 24 h levels were lower than the limit of detection.

below the limit of quantitation were also observed in the brain through 6 h. DHPG-Q^+ was associated with release of the parent compound in blood, with observed levels between 1.35 and 0.33 μg/ml. These are significantly below the ganciclovir level generated after ganciclovir treatment. By contrast, significantly higher levels of ganciclovir were generated in the brains of rats treated with DHPG-CDS than were generated in the brains of rats treated with ganciclovir. The levels were sustained, with 15-min and 6-h concentrations being almost identical (0.69 versus 0.66 μg/g), indicating potentially therapeutic concentrations throughout the 6-h time course (14). The area under the brain drug concentration curve from 0 to 6 h was five times greater after DHPG-CDS treatment (3.61 μg · h/ml) than after ganciclovir administration (0.664 μg · h/ml). Levels of ganciclovir in the blood were significantly lower after DHPG-CDS treatment than after ganciclovir administration (area under the curve from 0 to 6 h = 10.46 μg · h/ml after ganciclovir treatment and 1.418 μg · h/ml after DHPG-CDS treatment). The increased levels in the brain and decreased concentrations in blood resulted in an increase of the brain-to-blood drug concentration ratio, as measured by comparisons of the area under the curve, from 0.063 in the case of ganciclovir administration to 2.54 in the case of DHPG-CDS administration. This represents a 40-fold increase in the brain/blood drug concentration ratio. All tissue were cleared of drug by 24 h.

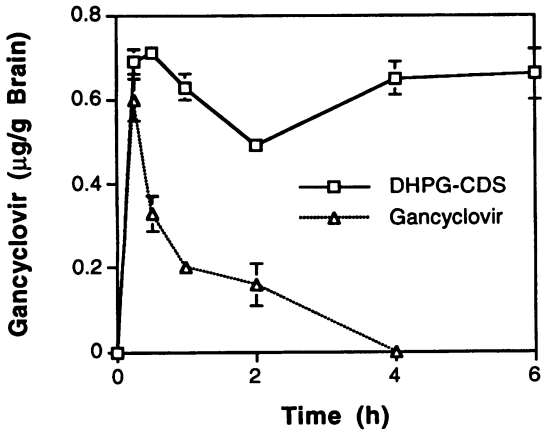

FIG. 3. Brain ganciclovir concentrations as a function of time after an intravenous dose of 80 μmol of either DHPG-CDS or ganciclovir per kg.

DISCUSSION

Human CMV infection is a pernicious opportunistic malady associated with immunosuppression with manifestations in the eye, brain, and lung. While very positive results can be pointed to in alleviation of retinitis, the treatment of encephalitic cytomegalic disease is far less successful with ganciclovir. Thus, Fiala et al. found that while a few patients and specifically those diagnosed with meningoencephalitic disease demonstrated some improvement with ganciclovir, the majority of patients with CNS CMV infection including subacute encephalopathy and polyneuropathies did not improve (13). In addition, some reports indicate that patients became worse when ganciclovir was used to treat brain infections (22).

The inability of ganciclovir to adequately treat central infection is likely related to its inability to penetrate biological membranes. As reviewed by Greig, there are three important factors governing CNS uptake of drugs, including the time-dependent free concentration profile of the compound of interest in plasma; the permeability of the compound through the blood-brain barrier, which is highly correlated with the derivative's lipophilicity or octanol-water PC; and the local blood flow (17). In the case of compound lipophilicity, studies have indicated that compounds with PCs of 1.0 (log PC > 0) are readily transferred across the blood-brain barrier (in the absence of enzymatic instability or other complicating factors) and their uptake is blood flow dependent. PCs of less than 0.1 (log PC < −1.0) are associated with permeability-limited uptake of compounds from brain to blood. Between these values, both blood flow and drug permeability may be limiting. The log PC value of ganciclovir is −2.0, which, as expected, confers poor (nonfacilitative) membrane penetration characteristics. As a result, the oral availability of ganciclovir is low (2.5 to 6%) and its ability to penetrate the blood-brain barrier is very limited. Autopsy studies indicate that ganciclovir concentrations in the brain were only 38% of the levels in cardiac blood and many times lower than the level of drug found in the kidneys (34). Other studies indicated poor uptake of ganciclovir into cerebrospiral fluid (31%) 3.5 h after intravenous infusion (15).

Various attempts have been made to improve the physicochemical properties of ganciclovir, with the most often stated aim of improving oral bioavailability or skin penetration. Thus, Martin et al. prepared a series of diester, ester-ether, and diether prodrugs of ganciclovir and tested the oral effectiveness of these compounds in a murine herpes encephalitis model (27). None of the compounds were dramatically more effective than ganciclovir, with 50% effective doses ranging between 55 and 125% of that for ganciclovir. The dihemisuccinate prodrug of ganciclovir was prepared to improve the

aqueous solubility of the parent compound and was in fact 13 times more water soluble (3). Unfortunately, the esters cleaved very slowly in plasma, precluding the use of this compound as a prodrug. The dipropyl derivative of ganciclovir was identified as a possible candidate for development, with a modestly improved (42%) oral bioavailability over that of ganciclovir, but further work demonstrated that its stability in aqueous solutions was poor (32). The diadamantane ester of ganciclovir was suggested by Powell et al. as a derivative with improved skin-penetrating properties (32). The prodrug exhibited good lipophilicity, solution stability, and esterase conversion to the parent compound. Other recently described ester prodrugs include aminomethylbenzoates, which combine such desirable properties as high water solubility at acid pH values with favorable log PC values and rapid enzymatic hydrolysis (20). Diether derivatives, especially the diisopropyl ether (HOE 602), appear to be useful prodrugs for enhancing oral bioavailability of ganciclovir (37, 38). HOE 602 was shown to increase the number of mice surviving a murine CMV challenge when the drug was dosed orally, and the prodrug was found to increase the area under the plasma ganciclovir concentration curve, compared with that of oral ganciclovir, by almost four times in monkeys (38). None of the derivatives or prodrugs developed, however, were tailored to enhance or sustain delivery to the brain.

Many of the derivatives mentioned above could enhance drug movement into the CNS, if so applied, on the basis of their increased lipophilicity, but this action is not selective. As a result, the extraction of the lipophile by other tissues is also increased relative to that of the unmanipulated drug (36). For toxic or cytotoxic agents this is undesirable since the prodrug often generates a greater tissue burden which often increases the toxicity of the prodrug. In attempting to dissociate these two parameters, we applied the redox-based CDS described by Bodor and colleagues to the delivery of ganciclovir to the brain (5, 6, 8).

The CDS approach requires that a molecular targetor be attached to the drug of interest. Ganciclovir has several potential sites for attachment of the targetor, including the two hydroxymethyl positions as well as the 3-hydroxy (keto-enol) function. While all of the sites mentioned are accessible, the initial design process suggested attachment of a single 1-methyldihydronicotinate targetor group to one of the hydroxymethyl groups. It was expected that this manipulation would provide a sufficient improvement in lipophilicity to allow for blood-brain barrier transit of the conjugate. Other manipulations were not attempted at the onset since they were thought to complicate the kinetic scheme. The designed CDS was prepared by established procedures and tested in vitro and in vivo to assay its ability to act as a brain-targeting delivery form.

The DHPG-CDS was found to be 55 times more lipophilic than the parent compound but still have a log PC value close to zero. This would suggest CNS uptake greater than that of ganciclovir but still partially dependent on molecular permeability (17). The corresponding quaternary salt was two- to threefold more hydrophilic than ganciclovir, consistent with its polar structure. This lower log PC value should aid in the rapid disposition of the DHPG-Q^+ which forms in situ from the administered DHPG-CDS. Buffer studies demonstrated good chemical stability of the compounds at neutral pH values. DHPG-CDS is poorly stable in the acid pH range, suggesting that oral delivery systems will require enteric coating to ferry the drug past the stomach to the intestine. DHPG-Q^+, on the other hand, is poorly stable in alkaline solutions because of ester hydrolysis. In rat brain homogenate, DHPG-CDS rapidly oxidized to the corresponding quaternary salt, with a $t_{1/2}$ of minutes, while the stability in rat blood lasted approximately 1 h. This suggested reasonable stability in blood during the distributional phase of the CDS and rapid conversion of the compound in brain. The $t_{1/2}$ of hydrolysis of the oxidized salt in brain homogenate was approximately 30 min and suggested facile conversion of the quaternary depot form to the parent agent.

Animal distribution data were supportive of the CDS concept in the case of DHPG-CDS. While uptake of ganciclovir was limited and its concentrations were readily eliminated from the brain, DHPG-CDS provided for sustained brain delivery of ganciclovir through 6 h at concentrations that have been reported to inhibit viral replication in vitro (50% inhibitory concentration = 0.5 to 3 μM; 6-h concentration in the brain = 2.7 μM) (14). The area under the brain drug concentration curves were fivefold higher after DHPG-CDS administration than after ganciclovir administration. The higher levels of ganciclovir in the brain may result from a distributional advantage offered by DHPG-CDS; i.e., the CDS may provide for a partitioning of the ganciclovir into brain compartments deeper than those that are accessible to the unmanipulated drug. Such a pharmacokinetic change would provide for a longer residence time even in the absence of high levels of the DHPG-Q^+ precursor. In addition, levels of the parent drug in blood after DHPG-CDS treatment were much lower than those generated by ganciclovir. The lower systemic levels may have important toxicological ramifications. The most often reported dose-limiting side effect of ganciclovir is neutropenia, which affects about 40% of patients taking the drug, followed by thrombocytopenia, which occurs in approximately 20% of all patients taking ganciclovir (12). The lower peripheral levels of ganciclovir produced by DHPG-CDS may therefore reduce the incidence or severity of these hematological reactions.

In summary, DHPG-CDS provides for some improvement in organ selectivity by producing a significant increase in the brain-to-blood and other organ-to-blood drug level ratios as a function of time. DHPG-CDS was found to sustain drug levels in the brain at therapeutically significant levels and to reduce concentrations in the blood compartment, which is associated with toxicity.

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