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(±)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)-cyclobutyl]guanine [(±)-BHCG or SQ 33 054]: a potent and selective inhibitor of herpesviruses

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Summary

(±)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine [(±)-BHCG or SQ 33 054] is a newly synthesized nucleoside analog with potent and selective antiviral activity against members of the herpesvirus group, including human cytomegalovirus. The activity against a thymidine kinase deficient HSV-2 mutant was 25-fold poorer than against the parent virus, suggesting that phosphorylation is an important prerequisite for antiviral activity against HSV-2. (±)-BHCG is readily phosphorylated by purified HSV-1 thymidine kinase, and BHCG triphosphate synthesized enzymatically is a selective inhibitor of HSV-1 DNA polymerase. (±)-BHCG did not inhibit host cell growth at concentrations at least 1000-fold higher than HSV-2 inhibitory concentrations. Subcutaneous administration of (±)-BHCG was protective against HSV-1 systemic infections in mice. BHCG is an exciting antiviral agent and represents a new class of nucleoside analogs.

(±)-BHCG; HSV-1; HSV-2

Introduction

The success of acyclovir as a safe and selective anti-herpesvirus agent has demonstrated that nucleoside analogs can be designed for minimal toxicity and potent viral inhibition (Straus et al., 1988). Furthermore, the selective antiviral ac-

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tivity observed at the levels of enzymatic activation and inhibition, cell culture protection and animal protection studies can be predictive of efficacy in the clinic. Ganciclovir, a compound anticipated to have clinical utility based on cell culture protection studies against human cytomegalovirus (HCMV) infections (Ashton et al., 1982; Field et al., 1983; Smee et al., 1983; Cheng et al., 1983; Plotkin et al., 1985) has been successfully used to suppress progressive HCMV retinitis in AIDS patients (Jacobson et al., 1988).

We have undertaken a program to identify novel and potentially useful anti-herpes virus nucleoside analogs, and here report on studies with SQ 33 054, (±)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine or (±)-BHCG which is a new, potent and selective inhibitor of herpesvirus replication in cell culture and in animals. Preliminary reports of the antiviral activities of (±)-BHCG were presented at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy (Braitman et al., 1989; Field et al., 1989; Slusarchyk et al., 1989).

Materials and Methods

Chemicals and enzymes

SQ 33 054, [(±)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine or (±)-BHCG] (Fig. 1), was synthesized at The Squibb Institute for Medical Research, Princeton, New Jersey according to methods described by Slusarchyk et al. (submitted for publication). Characterization of the final product was as follows: melting point >220°C; Analysis, calculated for $C_{11}H_{15}N_5O_3 \cdot 0.93\ H_2O$ (C, 46.85; H, 6.02; N, 24.84; H_2O, 5.93), Found (C, 46.49; H, 6.08; N, 24.45; H_2O, 5.93); HNMR (270 MHz, DMSO-d_6) δ 2.04 (2H, m), 2.36 (1H, m), and 2.68 (1H, m) [H-2′, H-3′, H-4′], 3.49 (4H, m, CH_2O), 4.42 (1H, ddd, each J = ca. 8.5 Hz, H-1′), 4.57 (1H, t, J = 5.3 Hz, OH), 4.62 (1H, t, J = 5 Hz, OH), 6.36 (1 H, s, NH_2), 7.82 (1H, s, H-8), 10.50 (1H, br, NH); ^{13}C NMR (67.94 MHz, DMSO-d_6) δ 156.85 (C-6), 153.28 (C-2), 150.91 (C-4), 135.71 (C-8), 116.76 (C-5), 63.46 and 61.41 (CH_2O), 47.65, 46.53, 33.05, 29.56; MS(FAB) 266 $(M + H)^+$, 264 $(M - H)^-$; UV (phosphate buffer, pH 7.2), λmax 253.3 nm (11 600), inflex 275 nm (8400). Inde-

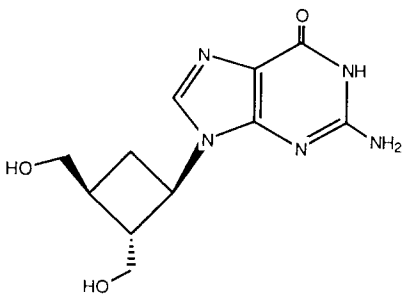

Fig. 1. (±)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine [(±)-BHCG or SQ 33 054].

pendently, Norbeck et al., 1989 and Hayashi et al., 1989 have disclosed preliminary studies on this compound (A69992).

Acyclovir was prepared as described in Netherlands Patent 7709458 (1978) issued to The Wellcome Foundation Limited, London. Ganciclovir, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine was prepared as reported by Martin et al. (1983). Acyclovir triphosphate (Germershausen et al., 1983; Larsson et al., 1986) was prepared from acyclovir in 14% yield according to the general procedure of Ludwig (1981). Acyclovir triphosphate was isolated as the triethylammonium salt by DEAE-cellulose chromatography and converted to the tetrapotassium salt by passage through a Dowex-50 (potassium form) column.

Guanylate kinase, nucleoside 5′-diphosphate kinase, creatine kinase and DNAse I activated calf thymus DNA were purchased from Sigma, St. Louis, MO. Activated calf thymus DNA was deproteinized with Proteinase K followed by phenol extraction before use. Nucleoside triphosphates (dGTP, dATP, dTTP and dCTP) were obtained from Pharmacia (Piscataway, NJ) and phosphocreatine from Boehringer-Mannheim (Indianapolis, IN). [Methyl-^{3}H]dTTP (21 Ci/mmol), and [α-^{32}P]dCTP (800 Ci/mmol) were obtained from New England Nuclear (Boston, MA).

Viruses, cells and media

HSV-1 strains Schooler, KOS and KOS(PFA^{R}-1) (the latter kindly provided by Dr. Y.-C. Cheng, University of North Carolina; Derse et al., 1982) as well as HSV-2 strains Curtis and 186 were prepared as extracts from infected VERO cell cultures. HCMV strain AD169 and VZV strain Ellen were prepared as suspensions of infected WI-38 cells. HSV-2 (186, 2′NDG^{R}) virus is thymidine kinase (TK) deficient and was previously described by Terry et al. (1988). MCMV, Smith strain, (ATCC VR-194) was passed in secondary mouse embryo cells. Vaccinia strain CL (ATCC VR-117), and influenza A, strain WSN (ATCC VR-219) were also obtained from ATCC. All virus stocks were stored at −70°C.

WI-38 (CCL75), VERO (CCL81), HeLa S3 (CCL2.2) and MDCK (CCL34) were obtained from ATCC and were grown in Eagle's minimum essential medium with Earle's salts (EMEM) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% FBS (Gibco Laboratories, Grand Island, NY).

Plaque reduction assay

Viruses were adsorbed to cell culture monolayers in 6-well culture plates (Costar, Cambridge, MA) for 1–2 h prior to addition of maintenance medium containing duplicate dilutions of the test compound (EMEM plus supplements, 1% carboxymethyl cellulose, 2.5% FBS ± drug). Inhibition of plaque development for all viruses was evaluated on monolayers stained after 4 to 6 days incubation at 37°C. ED_{50} values were determined from the drug concentration which conferred at least a 50% plaque reduction compared to virus controls. All titrations were done in duplicate and expressed as the range in repeat assays. HSV-1, HSV-2, HCMV, VZV, and vaccinia were assayed on WI-38 cell monolayers; influenza was assayed

on MDCK cell monolayers; MCMV was assayed on secondary mouse embryo cell monolayers.

Cell growth inhibition studies

WI-38 cells were plated at 1.2×10^5 cells per well in 12-well Costar plates containing 2 ml of growth medium. Following overnight incubation at 37°C, the cultures were refed with fresh growth medium containing serial dilutions of acyclovir, ganciclovir, (±)-BHCG, or no drug and incubation was continued at 37°C for an additional 3 days. Quadruplicate cultures for each concentration of drug evaluated were harvested by trypsinization and counted daily for viable cells by staining with trypan blue. Untreated control cell cultures increased approximately 3- to 5-fold.

Rates of phosphorylation of acyclovir and (±)-BHCG

TK was purified from HSV-1 (KOS) infected HeLa S3 cells. Cells were lysed (Terry et al., 1988) and applied to DEAE-cellulose in 0.35 M KPO_4, pH 7.4, 1 mM DTT, 50 mM KCl, 0.4 mM EDTA, 10% glycerol. The unbound material was dialyzed overnight against 20 mM Tris HCl, pH 7.6, 50 mM NaCl, 1 mM DTT, 2 mM EDTA, 10% glycerol and applied to a denatured DNA-cellulose column (Pharmacia) equilibrated in the same buffer. The unbound material was applied to a thymidine-agarose affinity column (Lee and Cheng, 1976) and eluted according to Fyfe (1982). SDS-PAGE indicated a single protein band with a molecular weight of 43000. TK was dialyzed versus 20 mM Tris-HCl, pH 7.5, 2 mM DTT, 1 mM ATP, 50% glycerol and stored at −80°C. A unit of TK is defined as the phosphorylation of 1 nmol of dThd per hour at 37°C in the buffer described below.

Phosphorylation of acyclovir and (±)-BHCG with HSV-1 thymidine kinase was determined under the following conditions: 50 mM Tris HCl, pH 7.5, 5 mM $MgCl_2$, 30 mM KCl, 2 mM DTT, 2.5 mM KF, 0.5 mM nucleoside, 5 mM ATP, and 0.075 mg/ml BSA, and 3U thymidine kinase at 37°C for 0–6 h. Samples were stored frozen at −20°C for subsequent analysis.

Reaction products were resolved by HPLC on a Whatman Partisil 5 ODS 3RAC column and eluted with 0–35% acetonitrile gradient in 10 mM ammonium phosphate/2 mM tetrabutylammonium phosphate. Nucleoside and nucleoside monophosphates were identified by retention time and ultraviolet spectrum utilizing a LKB Rapid Spectral Detector.

Enzymatic synthesis of BHCG triphosphate

BHCG triphosphate was prepared by incubating 5 mg/ml (±)-BHCG with HSV-1 thymidine kinase cloned and expressed in *E. coli*, in 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 30 mM KCl, 2 mM DTT, 2.5 mM KF, 5 mM ATP, 15 mM creatine phosphate, 2 U/ml guanylate kinase, 8 U/ml creatine kinase, and 0.075 mg/ml BSA at 37°C for 24 h. Additional thymidine kinase (20 U/ml), guanylate kinase (1 U/ml), creatine kinase (8 U/ml), and nucleoside 5′-diphosphate kinase (8 U/ml) were added

and incubation continued for 48 h at 37°C. The reaction products were separated by HPLC using a Synchropak AX200 column with a linear gradient of 0–100% 0.5 M KH_2PO_4, pH 5.6. Phosphate buffer was removed by chromatography on DEAE-Sephadex with 0.2–1.5 M triethylammonium acetate (pH 7.5) and repeated lyophilizations. The concentration of BHCG triphosphate was determined by UV spectroscopy ($\epsilon_{252\ nm}$ = 11600). NMR analysis of BHCG monophosphate indicated that the pseudo-5′-hydroxyl was the position of phosphorylation by thymidine kinase.

DNA polymerase inhibition studies

HSV-1 (Schooler) infected HeLa S3 cells were infected with a multiplicity of infection (MOI) of 10 PFU per cell, harvested 20 h post infection, and extracts prepared as described by Terry et al. (1988). The extract was clarified by centrifugation at 125000 × *g* for 1 h and adjusted to 0.4% NP-40. Aliquots were frozen in dry ice and stored at −70°C. The protein concentration was 5.0 mg/ml as determined by Coomassie Blue protein assay (Bradford, 1976). Uninfected HeLa S3 cell extracts were prepared in an identical manner and had a protein concentration of 2.4 mg/ml.

Inhibition of HSV-1 DNA polymerase by BHCGTP or ACVTP was determined in: 50 mM Tris-HCl, pH 8; 5 mM $MgCl_2$; 1 mM DTT; 0.1 M ammonium sulfate; 5 μM (each) dATP, dCTP and [^{3}H]dTTP (500 cpm/pmol); 30 μg/ml activated calf thymus DNA; 0.1 mg/ml BSA; HSV-1 (Schooler) infected HeLa extracts; and varying dGTP. Incubation was for 30 min at 37°C. Incorporation of [^{3}H]dTTP into DNA was quantitated by TCA precipitation and scintillation counting.

Inhibition constants were determined for HeLa S3 DNA polymerase under the following conditions: 50 mM Hepes, pH 7; 5 mM $MgCl_2$; 1 mM DTT; 30 μg/ml activated calf thymus DNA; 0.1 mg/ml BSA; 5 μM (each) dATP, dCTP and [^{3}H]dTTP (540 cpm/pmol); varying dGTP; and HeLa S3 extracts. Incubation was for 45 min at 37°C and incorporation of radiolabel into DNA was quantitated by TCA precipitation and scintillation counting.

Inhibition of viral DNA synthesis

Quantitation of viral DNA synthesis was performed essentially as described by Gadler et al. (1984) and Terry et al. (1988). WI-38 cells in 96-well microtiter dishes were infected with HSV-1 (KOS) at an MOI = 2 PFU/cell, or mock-infected. After 1 h adsorption at 37°C, the inoculum was removed and replaced with media containing varying amounts of drug; six concentrations of each compound were tested in duplicate and incubation was continued for 18 h post infection. At that time cells were loosened with trypsin-EDTA and filtered through a nitrocellulose filter using a filtration manifold. Filters were processed and hybridization performed as described by Maniatis et al. (1982). The probe consisted of pNN3 plasmid DNA (obtained from Dr M. Challberg, NIH, Bethesda, MD) containing a 5.5 kb fragment of HSV-1 (KOS) DNA derived from the region encoding the HSV-1 DNA poly-

merase gene and located adjacent to the origin of replication for the unique long region of the herpes genome (Challberg, 1986). This probe was radiolabeled with [α-^{32}P]dCTP to a specific activity of 1×10^7 cpm/μg and the amount of radiolabel hybridized was quantitated by cutting the spots from the filter and counting them in a liquid scintillation counter.

Mouse protection studies

Female Swiss-Webster mice weighing 20–25 g were obtained from Taconic Farms, Germantown, New York. Mice were infected with an intraperitoneal injection of HSV-1 (Schooler), approximately 3×10^3 PFU/mouse. Antiviral therapy was initiated at 1 h post infection and continued twice daily for 5 days. Compounds in 0.5 ml PBS were administered subcutaneously. Animal survival was assessed daily for 21 days at which time remaining animals were terminated. PD_{50} values based on survival were calculated by the method of Reed and Muench (1938).

Results

In vitro antiviral activity and cytotoxicity

(±)-BHCG was evaluated for antiviral activity in plaque reduction assays against a spectrum of viruses. The results of these studies, in comparison to acyclovir and ganciclovir, are summarized in Table 1. We report that in these assays, (±)-BHCG is equivalent or superior to acyclovir against HSV-1 and HSV-2, and approxi-

TABLE 1

Antiviral efficacy of (±)-BHCG, acyclovir and ganciclovir in cell culture

Virus	ED_{50}, μM		
	(±)-BHCG	Acyclovir	Ganciclovir
HSV-1 (Schooler)	0.08–0.2	0.4–0.8	0.02–0.04
HSV-1 (KOS)	0.4–0.8	0.4–0.8	0.04–0.08
HSV-1 (KOS, PFA^R-1)	0.4–0.8	2–4	0.04–0.08
HSV-2 (Curtis)	0.2–0.4	0.8–2	0.2–0.4
HSV-2 (186)	0.04	0.2–0.4	0.04
HSV-2 (186,2'NDG^R)	1–2	444	20–40
HCMV (AD169)	2–4	20–40	0.4–0.8
MCMV (Smith)	1–2	2–4	4
VZV (Ellen)	0.2	2–4	0.8–2.0
Vaccinia (CL)	190	440	100–200
Influenza A (WSN)	>377	440	>400

Plaque assays for HSV-1, HSV-2, HCMV, vaccinia and VZV were performed with WI-38 cell monolayers. HSV-1 (KOS, PFA^R-1) is resistant to phosphonoformic acid (Derse et al., 1982) and has an altered viral DNA polymerase (Cheng et al., 1983). HSV-2 (186,2'NDB^R) is deficient in thymidine kinase activity and was described by Terry et al. (1988).

TABLE 2

Phosphorylation rates and extents of (±)-BHCG[a]

	Initial Rate, μM/h	Relative extent[b]
Acyclovir	15	1.0
(±)-BHCG	42	2.2
dThd	250	–

[a]Nucleosides (0.5 mM) were phosphorylated by HSV-1 thymidine kinase in quadruplicate, to determine initial reaction rates. Reaction products were resolved and quantitated by HPLC.
[b]Phosphorylation extents (3 determinations) were measured after 23 h incubation at 37°C. Under these conditions, dThd was completely phosphorylated by 5 h.

mately equivalent to ganciclovir against HSV-2. In addition, (±)-BHCG is active against the five herpes group viruses tested, including HCMV and VZV, but poorly active against vaccinia, an unrelated DNA virus, and inactive against influenza virus.

The ED_{50} for (±)-BHCG is substantially increased (at least 25-fold) against an HSV-2 TK deficient variant, suggesting that phosphorylation by viral TK influences the activity against HSV-2. Like ganciclovir, the ED_{50} of (±)-BHCG against the DNA polymerase mutant PFA^R-1 is unaltered compared to wild-type HSV-1 KOS. This is in contrast to the decreased efficacy of acyclovir against this mutant.

(±)-BHCG was also evaluated for inhibition of growth of WI-38 cells for 3 days. Repeated dose response curves demonstrated that the ED_{50} for cytostatic activity varied widely between 48 and 380 μM (avg = 190 μM). Based on the ED_{50} of 0.04 μM for inhibition of HSV-2 (strain 186) plaque formation, the calculated in vitro therapeutic index ranged from 1200 to 9400 (avg = 4760). In contrast, the calculated therapeutic index for ganciclovir was 9800–12000, and that for acyclovir was greater than 2000. For all three compounds the proportion of viable cells, as determined by dye exclusion, was unaltered for the duration of the experiment.

Phosphorylation of (±)-BHCG and inhibition of DNA polymerases

As shown in Table 2, (±)-BHCG is phosphorylated by HSV-1 TK to twice the extent and at approximately three times the rate for acyclovir. While these results

TABLE 3

Inhibition of HSV-1 and HeLa DNA polymerases

	Ki, μM	
	HSV-1	HeLa
Acyclovir triphosphate	0.013 ± 0.004	1.3
BHCG triphosphate	0.004 ± 0.002	2.2

Summary of inhibition constants (± SD) determined for acyclovir triphosphate (N = 5, N = 2) and BHCG triphosphate (N = 8, N = 3) for HSV-1 DNA polymerase and HeLa DNA polymerase activities, respectively.

are not unexpected, based on the TK-dependence demonstrated for efficacy of (±)-BHCG against HSV-2 in cell culture studies (Table 1), they must be viewed with caution. (±)-BHCG was prepared as a racemic mixture and HSV-1 TK may not phosphorylate both enantiomers at the same rate (Karkas et al., 1987). Final determinations of the rate and extent of phosphorylation require studies using the separate enantiomers.

The triphosphates of BHCG and acyclovir were prepared enzymatically as described in Materials and Methods. As shown in Fig. 1, BHCG triphosphate is a competitive inhibitor with respect to dGTP in both the HSV-1 and HeLa DNA polymerase assays. The inhibition constants are summarized in Table 3. BHCG triphosphate is about 3-fold more potent an inhibitor of HSV-1 DNA polymerase than is acyclovir triphosphate. Against HeLa DNA polymerase alpha, BHCG triphosphate was a less potent inhibitor than acyclovir triphosphate. Taken together,

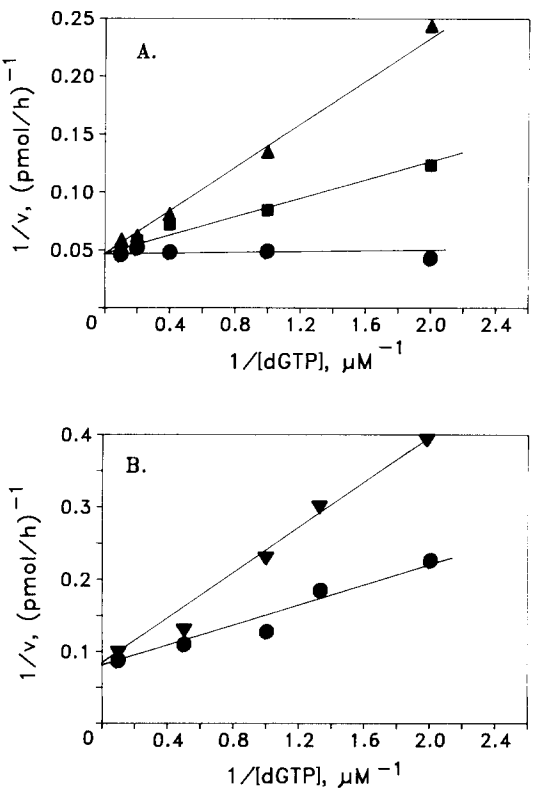

Fig. 2. Inhibition of DNA polymerases by BHCG triphosphate. (A) HSV-1 DNA polymerase activity was assayed with HSV-1 (KOS) infected HeLa extracts in 50 mM Tris-HCl, pH 8; 5 mM $MgCl_2$; 1 mM DTT; 0.1 M $(NH_4)_2SO_4$; 5 μM (each) dATP, dCTP, [^{3}H]dTTP (500 cpm/pmol); 30 μg/ml activated calf thymus DNA; 0.1 mg/ml BSA; and varying dGTP. BHCG triphosphate concentrations were: (●) 0 μM, (■) 0.1 μM, (▲) 0.2 μM. (B) HeLa DNA polymerase activity was assayed as described in Materials and Methods. (●) 0 μM and (▼) 2 μM BHCG triphosphate.

TABLE 4

Comparison of antiviral efficacy in cell culture by inhibition of HSV-1 DNA synthesis

Antiviral compound	ED_{50}, μM*	
	DNA hybridization	Plaque reduction
(±)-BHCG	0.008–0.08	0.4–0.8
Acyclovir	0.04–0.08	0.4–0.8
Ganciclovir	<0.004	0.04

*Determined using HSV-1 (KOS) infection of WI-38 cells as defined in Materials and Methods.

these studies suggest that BHCG triphosphate is slightly more selective than acyclovir triphosphate as an inhibitor of virus DNA polymerase. BHCG triphosphate is an uncompetitive inhibitor of dATP and dTTP incorporation into calf thymus DNA (data not shown). These results suggest that at least one of the mechanisms of action of BHCG is inhibition of viral DNA polymerase by BHCG triphosphate.

Viral DNA synthesis

The effects of (±)-BHCG, acyclovir and ganciclovir on viral DNA replication were measured directly by nucleic acid hybridization. This technique yields ED_{50} values based on inhibition of viral DNA accumulation in HSV-1 infected cells and is generally more sensitive than the plaque reduction assay (Terry et al., 1988). As shown in Table 4, (±)-BHCG is a potent inhibitor of viral DNA synthesis, with an ED_{50} equal or less than that for acyclovir, but greater than that for ganciclovir.

TABLE 5

Efficacy of BHCG and acyclovir against HSV-1 intraperitoneal infection

Test compound	Dose (mg/kg/day)	Survival (alive/total)	PD_{50} (mg/kg)	Mean day of death for total dead ± (SD)
(±)-BHCG	50.0	8/10*	20	11.0 ± 5.7
	12.5	3/10		12.3 ± 3.1**
	3.1	0/10		9.8 ± 3.4
	0.8	1/10		9.0 ± 2.4
Acyclovir	50.0	4/10	43	11.8 ± 4.0
	12.5	2/10		10.3 ± 3.1
	3.1	1/10		9.8 ± 3.2
	0.80	0/10		7.1 ± 1.3
Placebo		1/20		8.1 ± 1.3

Mice were infected with 3×10^3 PFU HSV-1 (Schooler) (i.p.) and treated (s.c.) twice daily for 5 days with drug or placebo. Animal survival was assessed daily for 21 days at which time remaining animals were terminated.

*Significantly different from placebo, $P<0.05$, Fisher's exact test.

**Significantly different from placebo, $P<0.05$, Student's t-test.

These results are consistent with the relative plaque reduction ED_{50} values and support the suggested mechanism of inhibition of viral DNA synthesis.

Mouse protection studies

The capacities of (±)-BHCG and acyclovir to protect animals against fatal HSV-1 infection are shown in Table 5. (±)-BHCG and acyclovir are efficacious in this model with PD_{50} values of 20 mg/kg/day and 43 mg/kg/day, respectively. (±)-BHCG significantly decreased mortality when given at 50 mg/kg/day with 80% of the animals surviving compared to 5% of placebo animals. (±)-BHCG also extended survival time. For example, animals given 12.5 mg/kg/day showed significantly extended survival time, as indicated by the mean day of death of 12.3 ± 3.1 days compared to the placebo group which had a mean of 8.1 ± 1.3 days. Animals treated with acyclovir had a decrease in mortality and extended survival times, but the acyclovir treated groups were not statistically different from the placebo group.

Discussion

In this report we have described (±)-BHCG, a representative of a new class of nucleoside analogs with both potent and selective activity against the herpesviruses. (±)-BHCG inhibited HSV-1, HSV-2, and VZV plaque development in cell culture at concentrations of less than 1 μM and was active against HCMV at 2–4 μM. The pattern of inhibition of the viruses evaluated indicates that (±)-BHCG has an antiviral profile similar to that of ganciclovir. The ability of (±)-BHCG to afford protection against a fatal systemic HSV-1 infection in mice is consistent with its potent activity in cell culture.

The selectivity of (±)-BHCG is demonstrated by its poor antiviral activity against vaccinia virus, lack of antiviral activity against influenza virus, and relative inability to inhibit host cell growth at concentrations inhibitory to herpesvirus replication. Furthermore, based on the 25- to 50-fold increase in ED_{50} against a TK deficient mutant of HSV-2, it is apparent that the antiviral activity of (±)-BHCG against HSV is at least partially dependent on phosphorylation by viral TK. The potent competitive inhibition of HSV DNA polymerase by BHCG triphosphate suggests that the mechanism of antiviral action of BHCG is through inhibition of this enzyme. Indeed, the selectivity for inhibition of viral DNA polymerase compared to inhibition of cellular DNA polymerase, coupled with some viral TK dependence for phosphorylation would appear to account for the selective antiviral activity at nontoxic concentrations.

Extensive mode-of-action studies, animal protection and bioavailability studies are currently being completed for (±)-BHCG and will be the subjects of future reports. It should be emphasized that (±)-BHCG is a racemic mixture. The ED_{50} values for plaque inhibition by each enantiomer, their respective rates of phosphorylation, and the relative capacities of each enantiomer-triphosphate to inhibit DNA polymerases will be the subject of extensive investigations.

At this juncture (±)-BHCG can be considered an exciting new anti-herpesvirus compound representing a new class of nucleoside analogs. Its potential for treatment of herpesvirus infections, particularly those due to cytomegaloviruses is being vigorously pursued.

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