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9-{[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine: A selective inhibitor of herpes group virus replication

(antiviral activity/human cytomegalovirus/oral efficacy/herpes simplex virus 1 and 2/viral thymidine kinase)

A. K. FIELD*, M. E. DAVIES*, C. DEWITT*, H. C. PERRY*, R. LIOU†, J. GERMERSHAUSEN†, J. D. KARKAS†, W. T. ASHTON‡, D. B. R. JOHNSTON‡, AND R. L. TOLMAN‡

*Department of Virus and Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486; and †Department of Biochemistry and ‡Department of Synthetic Organic Chemistry, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

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ABSTRACT **9-{[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine (2′-nor-2′-deoxyguanosine; 2′NDG) selectively inhibits the replication of herpes group viruses. In cell culture studies 2′NDG was at least 10-fold more potent than acyclovir (ACV) in inhibition of human cytomegalovirus replication and Epstein–Barr virus-induced lymphocyte transformation and was about as effective as ACV in inhibition of herpes simplex viruses 1 and 2 and varicella zoster virus. Orally administered 2′NDG was 6- to 50-fold more efficacious than ACV in treating systemic or local HSV-1 infection or HSV-2 intravaginal infection in mice. The mode of action of 2′NDG appears to involve phosphorylation by herpes simplex virus thymidine kinase and subsequent phosphorylations by cellular kinases to produce 2′NDG triphosphate, which is a potent inhibitor of herpes virus DNA polymerase. Compared to ACV, 2′NDG was a more efficient substrate for HSV-1 thymidine kinase (V_{max}/K_m for 2′NDG 30-fold higher than that for ACV), whereas 2′NDG monophosphate is a more efficient substrate for GMP kinase (V_{max}/K_m for 2′NDG monophosphate 492-fold higher than that for ACV monophosphate). The combined effect is more rapid production of the inhibitory triphosphate from 2′NDG than from ACV.**

As part of our studies on the structure–activity relationships of herpes virus encoded thymidine kinase (TK) and DNA polymerase, a nucleoside analog, 9-{[2-hydroxy-1-(hydroxymethyl)-ethoxy]methyl}guanine (2′-nor-2′-deoxyguanosine; 2′NDG) (1–3) was synthesized. 2′NDG is an efficient substrate for the herpes simplex virus 1 (HSV-1) TK and is readily converted to the triphosphate, a potent inhibitor of viral DNA polymerase (1).

In the present studies, a chemical synthesis of 2′NDG, the characteristics of its selective phosphorylation by HSV-1 TK, and its rapid conversion to the triphosphate are more fully described. In addition, data are presented demonstrating that the rapid phosphorylation of 2′NDG is correlated with potent inhibition of herpes virus replication in cell cultures and both prophylactic and therapeutic efficacy against herpes virus infections in mice.

MATERIALS AND METHODS

Materials. Phosphocreatine, creatine kinase, ATP, deoxythymidine, and dGMP were purchased from Sigma; GMP kinase (hog brain) and NADH, from Boehringer Mannheim; lactate dehydrogenase, from Worthington; [*methyl*-^{3}H]deoxythymidine, from New England Nuclear. 9-(2-Hydroxyethoxymethyl)guanine (acyclovir; ACV) and [^{14}C]ACV (labeled in purine) were synthesized at Merck Sharp & Dohme Research Laboratories by methods of Schaeffer (4, 5). HSV-1 strains Schooler, S., McIntyre, and McKrae and HSV-2 strain Curtis were prepared as 10% freeze–thaw extracts from infected cell cultures. Epstein–Barr virus (EBV) stocks were prepared from media from B_{95-8} lymphoblastoid cell cultures. Varicella zoster virus (VZV) and human cytomegalovirus (HCMV), strains Towne and AD_{169}, were kindly provided by B. J. Neff (Dept. of Virus & Cell Biology, Merck Sharp & Dohme Research Laboratories). Mengo and vaccinia viruses were obtained from the Merck virus collection. All virus stocks were stored at −70°C.

Primary rabbit kidney cell cultures were prepared by standard procedures from 2- to 3-week-old New Zealand White rabbits and grown in Eagle's minimal essential medium (GIBCO) plus 5% fetal calf serum. MRC-5 diploid human cells were grown in Eagle's basal medium (GIBCO) plus 10% fetal calf serum.

ICR/Ha mice were provided by Merck Sharp & Dohme. HRS/J mice were obtained from Buckshire Farms (Perkasie, PA).

***In Vitro* Antiviral Assays.** ED_{50}s were calculated either by determining the drug concentration (μg/ml) required to confer 100% inhibition of virus-induced cytopathic effect in at least half the quadruplicate infected cultures (for HSV-1, HSV-2), or by determining the drug concentration (μg/ml) required to confer a 50% plaque inhibition on duplicate cell monolayers [for HSV-1, VZV, HCMV, Mengo virus, and vaccinia virus]. For both assays, the antiviral compound was added to the maintenance medium at the time of infection. Viral cytopathic effect was evaluated after incubation for 5 days at 37°C, and plaque development was evaluated after incubation for 3 days (7 days for HCMV) at 37°C.

Inhibition of EBV replication was measured by prevention of transformation of normal cord lymphocytes to lymphoblastoid cells. In brief, lymphocyte-rich suspensions were prepared from fresh, heparinized human cord blood specimens by differential centrifugation through lymphocyte separation medium (LSM; Litton Bionetics). The lymphocyte-rich layer was washed three times and resuspended in RPMI medium 1640 (GIBCO) containing 20% fetal calf serum at 5×10^5 cells per ml. Duplicate cell cultures were infected in the presence of 2′NDG or ACV at 0, 1, 5, 10, 50, or 100 μg/ml and maintained for up to 40 days at 37°C. Transformation was determined by development of cell clumps (five or more cells), which correlated with development of EBV nuclear antigen, increased DNA synthesis, and eventual establishment of lymphoblastoid cell lines (data not shown).

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Abbreviations: 2′NDG, 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine or 2′-nor-2′-deoxyguanosine; ACV, 9-(2-hydroxyethoxymethyl)guanine or acyclovir; -MP, monophosphate; HSV-1, HSV-2, herpes simplex viruses 1 and 2; EBV, Epstein–Barr virus; HCMV, human cytomegalovirus; VZV, varicella zoster virus; TK, thymidine kinase; i.p., intraperitoneal; pfu, plaque-forming units.

***In Vivo* Antiviral Assays.** Parenteral infection of 20-g ICR/Ha mice was initiated by intraperitoneal (i.p.) injection of 0.5 ml of saline containing approximately 50 lethal doses (50 LD_{50}) of HSV-1 strain Schooler [750 plaque-forming units (pfu)]. Infected mice were observed daily for 15 days. Vaginal infection of 30-g ICR/Ha mice was accomplished according to the methods of Nahmias *et al.* (6). Mice were vaginally swabbed with a saline-soaked cotton swab and then infected with a cotton tampon carrying approximately 10 LD_{50} of HSV-2 (2×10^4 pfu). Tampons were removed after one day. Infected animals developed vaginitis after 4 days, followed by posterior paralysis and death after day 7. Statistical analyses were performed on survival times on each experiment according to the methods of Liddel (7). Parallel line analyses were performed on transformed survival times to generate dose–response curves and calculate relative potencies. Statistical significance was based on a 95% confidence interval. Orofacial infection of 20-g female HRS/J mice with HSV-1 strain S was accomplished by the method described by Klein *et al.* (8). Briefly, mice were abraded on the snout with a 20-gauge needle and infected by application of 0.05 ml of saline containing 2×10^5 pfu to establish a sublethal localized infection in all animals. Inflammation developed by day 5 and rapidly progressed to vesicles, which were evaluated at day 7. Orofacial lesion scores were compared for different treatment groups by Duncan analysis in which pairwise comparisons were performed at a level of significance of $P = 0.05$ (9). Parallel line analyses were performed on average lesion scores to determine dose–response curves and calculate relative potencies of 2'NDG and ACV. For all experiments, oral treatment of animals was achieved by 0.1 ml gavage of unanesthetized mice with drug dissolved in saline at pH 10.5–11.0.

Nucleoside Phosphorylation Studies. HSV-1 TK was isolated from HeLa cells infected with HSV-1 (Patton strain) at a multiplicity of infection of 10, by a modification of the procedure of Cheng and Ostrander (10). The active fractions from the deoxythymidine affinity column were concentrated as described by Fyfe *et al.* (11) and stored under liquid nitrogen. A HeLa cell TK was isolated in a similar manner from uninfected HeLa cells. One unit of enzyme catalyzes the formation of 1 nmol of dTMP per min at 37°C.

For the determination of the kinetics of phosphorylation of deoxythymidine, ACV, and 2'NDG by viral and HeLa cell TK, various concentrations of ^{3}H-labeled deoxythymidine, ^{14}C-labeled ACV, or ^{14}C-labeled 2'NDG were incubated with the enzyme at 37°C, and the extent of phosphorylation was determined by the DEAE filter disk method (11). The compositions of the assay mixtures are indicated in the legend of Table 2. The monophosphates of 2'NDG (2'NDG-MP) and ACV (ACV-MP) were prepared enzymatically with purified HSV-1 TK. The nucleoside analogue (1.5–2.5 mg/ml) was incubated at 37°C with 50 mM potassium phosphate buffer, pH 6.5/1 mg of bovine serum albumin per ml/5 mM ATP/5 mM $MgCl_2$/1 mM dithiothreitol/10 mM phosphocreatine/creatine kinase at 12.5 units per ml/2.5 mM NaF/50 units of purified HSV-1 TK per ml. The progress of the reaction was monitored by HPLC. The product was purified by preparative HPLC using an anion-exchange column (Micro Pac AX-10, Varian) and desalted by chromatography on DEAE-cellulose with triethylammonium carbonate, pH 7.6, as the eluting solvent. The fractions containing the product were freeze-dried and purity was confirmed by analytical HPLC.

The kinetics of phosphorylation of dGMP, ACV-MP, and 2'NDG-MP by GMP kinase were determined by a spectrophotometric assay coupling the phosphorylation to the pyruvate kinase–lactate dehydrogenase system and measuring the oxidation of NADH by absorption at 340 nm (12).

RESULTS

Synthesis of 2'NDG. See Fig. 1.

1-Chloro-2-acetoxymethoxy-3-acetoxypropane (II). A mixture of 4-chloromethyl-1,3-dioxolane (13) (**I**, 24.5 g, 0.2 mol), acetic anhydride (60 ml, 0.63 mol), glacial acetic acid (6.7 ml, 0.12 mol), and $ZnCl_2$ (2.0 g, 0.015 mol) was stirred under a nitrogen atmosphere in a 250-ml three-necked flask equipped with a thermometer. The temperature rose from 25°C initially to 54°C within 5 min. The mixture was stirred for a total of 2 hr at room temperature. The reaction was determined to be complete at that time by TLC on silica gel [hexane/ethyl acetate, 1:1 (vol/vol)]. After solvent removal under reduced pressure, the oil was mixed with diethyl ether (100 ml), washed with saturated $NaHCO_3$ solution and water, dried ($MgSO_4$), filtered, and concentrated under a stream of nitrogen, leaving 41.0 g of an oil as a mixture of isomers. TLC on silica gel [diethyl ether/petroleum ether, 1:1 (vol/vol)], R_f values of 0.67 and 0.56. After preliminary filtration through 20 g of silica gel in dichloromethane and concentration of the effluent, 30 g of the isomers was separated in two portions on a Waters Auto Prep 500 HPLC, two packs, flow rate 250 ml/min, using diethyl ether/petroleum ether, 1:3 (vol/vol), as the eluent. The first $3\frac{1}{2}$ column volumes provided, after concentration, 3.5 g of the minor isomer. The next $\frac{1}{2}$ column volume gave 7.2 g of mixed isomers, and a final column volume afforded 16 g of **II** (final yield, after resolution of mixed isomers, 85%). Proton magnetic resonance (ppm, relative to tetramethylsilane in deuterated dimethyl sulfoxide): 2.02 (s, 6H, OAc); 3.6–3.8 (m, 2H, C^1H); 4.0–4.2 (m, 3H, C^2H and C^3H); 5.3 (s, 2H, OCH_2O).

1,3-Diacetoxy-2-acetoxymethoxy propane (III). Anhydrous potassium acetate (35 g, 0.356 mol) was added to 35 g (0.156 mol) of **II** in 250 ml of dimethyl formamide, which was stirred well with a mechanical stirrer. The suspension was heated to 150°C and maintained there until the reaction was determined to be complete by TLC (diethyl ether/petroleum ether, 1:1), about 1.5 hr. The cooled reaction mixture was diluted with 1 liter of water and extracted three times with ethyl acetate.

The combined ethyl acetate extracts were washed with water and saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The resultant crude product could be used directly in the next step (approximately 90% yield). Purification of **III** for chemical and physical characterization was accomplished by silica gel chromatography using diethyl ether/petroleum ether, 1:1, as the eluent. Proton magnetic resonance (ppm, relative to tetramethylsilane in C^2HCl_3): 2.10 (br s, 9H, AcO); 4.18 [br s, 5H, $(OCH_2)_2CH$]; 5.35 (s, 2H, OCH_2O).

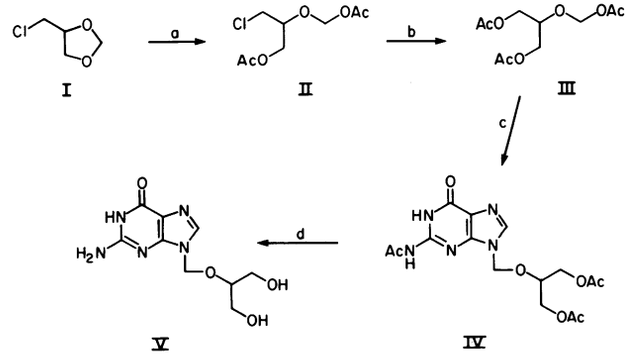

FIG. 1. Chemical synthesis of 2'NDG (**V**). a, $Ac_2O/ZnCl_2/HOAc$; b, KOAc/dimethylformamide; c, diacetylguanine/$C_2H_5SO_3H$; d, aqueous CH_3NH_2.

9-{[2-Acetoxy-1-(acetoxymethyl)ethoxy]methyl}-2-acetamidopurin-6-one (IV). A short-path distillation apparatus with a 500-ml distilling flask was charged with diacetylguanine (14) (43 g, 0.18 mol), **III** (49 g, 0.198 mol), and ethanesulfonic acid (0.6 g). The mixture was heated at 165–170°C under reduced pressure to remove acetic anhydride as it was formed in the fusion reaction. After 0.5 hr, the melt was allowed to cool, dissolved in dichloromethane (1 liter), and filtered. The solution was allowed to percolate through a silica gel column (350 g, packed in dichloromethane) and the products were eluted with dichloromethane (1.5 liter), dichloromethane/methanol, [98:2 (vol/vol), 1.5 liter], and dichloromethane/methanol [97:3, 8 liters] in turn. Fractions containing the slower of the two isomers as a major product were combined and evaporated to a foam (TLC monitor: dichloromethane/methanol, 96:4; silica gel). Dissolution in dichloromethane (10 ml/1.0 g) gave a clear solution from which the desired product crystallized, m.p. 173–174°C (33%). Proton magnetic resonance (ppm, relative to tetramethylsilane in deuterated dimethyl sulfoxide): 1.9 (s, 6H, AcO), 2.2 (s, 3H, AcN), 3.9–4.14 [m, 5H, $(OCH_2)_2CH$], 5.50 (s, 2H, OCH_2N), 8.16 (s, 1H, C^8H).

2'NDG (V). Aqueous methylamine [40% (wt/wt), 180 ml] was added to 18.0 g of **IV** and heated at 75°C for 1.5 hr. The solution was then allowed to cool to room temperature overnight, filtered, and evaporated to dryness under reduced pressure. The colorless solid was recrystallized from water (330 ml) with addition of acetic acid to pH 3.5 (1 ml). The product was filtered, washed with cold water, and air-dried to obtain 12.0 g of the monohydrate (95%), m.p. 248°C (decomposition). Reverse-phase HPLC on a Whatman C_8 column with water as the eluent showed a single peak. Proton magnetic resonance (ppm, relative to tetramethylsilane in deuterated dimethyl sulfoxide) 4.61 (t, J = 5 Hz, 1H, OH); 5.43 (s, 2H, OCH_2N); 6.45 (br s, 2H, NH_2); 7.71 (s, 1H, C^8H). UV (pH 7 buffer): λ_{max} 251 nm (ε, 14,700 M^{-1} cm^{-1}). [^{14}C]2'NDG was synthesized from [^{14}C]-guanine by the procedure described for the synthesis of **IV** and **V**. Satisfactory elemental analyses (C, H, and N) were obtained for all new compounds and intermediates.

Antiviral Activities of 2'NDG. As shown in Table 1, 2'NDG and ACV confer equivalent protection against HSV-1, HSV-2, and VZV infections in cell culture, and the efficacy did not vary substantially among the strains of HSV-1 tested. However, 2'NDG was at least 10-fold more active than ACV as an antiviral agent against HCMV and EBV. Against the Towne and AD_{169} strains of HCMV, the ED_{50} for 2'NDG was between 0.1 and 1.6 μg/ml, with means of 0.5 μg/ml and 0.8 μg/ml (six assays each), respectively. 2'NDG inhibited EBV-induced cord cell transformation, resulting in prevention of establishment of continuous lymphoblastoid cell cultures.

2'NDG given by subcutaneous injection was previously shown effective in protecting mice against i.p. HSV-1 infection resulting in encephalitis and death (1). In the present studies, 2'NDG was also effective when given orally in preventing HSV-1 systemic infection, HSV-1 orofacial infection, or HSV-2 vaginal infection in mice.

ICR/Ha mice infected with HSV-1 (i.p.) were treated twice daily by oral gavage with the indicated doses of 2'NDG or ACV for 7 days, starting immediately after infection (Fig. 2). Total protection, as measured by survival, was conferred by 50 mg of 2'NDG per kg per day. A comparable dose of ACV resulted in 20% survival. Significant increases in survival times compared to placebo-treated animals were caused by doses of 0.8 mg/kg per day for 2'NDG and 50 mg/kg per day for ACV. The potency of 2'NDG relative to ACV was 50.3, which was statistically significant.

Oral gavage with 2'NDG was also more effective against orofacial infection than was gavage with ACV. HRS/J hairless mice infected on the snout with HSV-1 were treated twice daily for 7 days starting 3 hr after infection (Fig. 3). Significant reduction in lesion development, determined on day 7, was stimulated by 0.2 mg of 2'NDG per kg, per day, compared to similar reduction using ACV at 12.5 mg/kg per day. The potency of 2'NDG relative to ACV was 6.9, which was statistically significant.

Oral gavage of mice vaginally infected with HSV-2 twice daily for 10 days starting immediately after infection resulted in a significant extension of survival time compared to placebo-treated animals at doses of 0.8 mg/kg per day (data not shown). By contrast, ACV conferred significant protection at 12.5 mg/kg per day. The potency of 2'NDG relative to ACV was 28.1, which was statistically significant. All the animals treated with 2'NDG at 50 mg/kg per day remained free of vaginitis for the duration of the experiment. In addition, although 30% of the animals treated with 12.5 mg of 2'NDG per kg per day developed

Table 1. Antiviral activities of 2'NDG and ACV *in vitro*

Virus	ED_{50}, μg/ml 2'NDG	ACV
HSV-1 (Schooler)	1–3 (0.25)*	1–3 (0.25)*
HSV-1 (S)	1–3*	ND
HSV-1 (McIntyre)	3*	ND
HSV-1 (McKrae)	1–3*	ND
HSV-2 (Curtis)	1–3*	1–3*
HCMV (Towne)	0.1–0.6†	2.2–17.7†
HCMV (AD169)	0.4–1.6†	25†
VZV (KMcC)	1–2†	1–2†
EBV (B_{95-8})	1–5‡	>10–100‡
Mengo	NA§	NA§
Vaccinia	NA§	NA§

NA, not active at 100 μg/ml; ND, not done.
* Determined as drug concentration to give 100% inhibition of viral cytopathic effect in half of primary rabbit kidney cell cultures. Values in parentheses were determined by plaque reduction on primary rabbit kidney cell monolayers.
† Determined by plaque reduction assay on MRC-5 cell monolayers.
‡ Determined as the concentration required to cause inhibition of transformation of human cord lymphocytes by EBV from B_{95-8}, marmoset lymphoblastoid cell cultures.
§ Determined for 2'NDG by plaque reduction assay on primary rabbit kidney cell monolayers. Results for ACV as published by Schaeffer *et al.* (5).

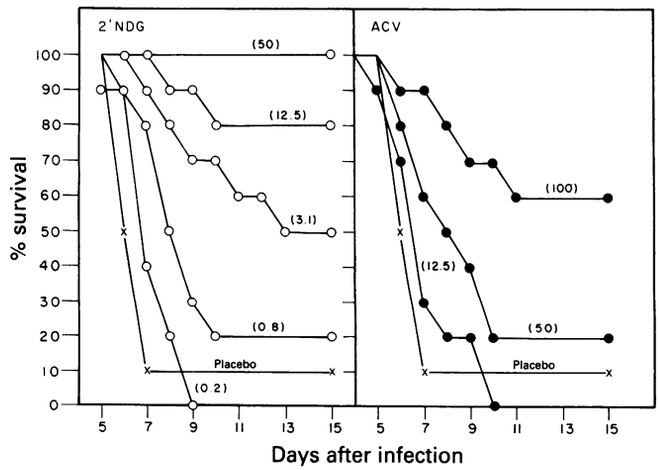

FIG. 2. Oral treatment of mice after i.p. HSV-1 infection. ICR/Ha mice (20 g) were infected and then treated twice daily for 7 days with 2'NDG or ACV using the dosage (mg/kg per day) indicated in parentheses. Dead and surviving mice were recorded each day for 15 days.

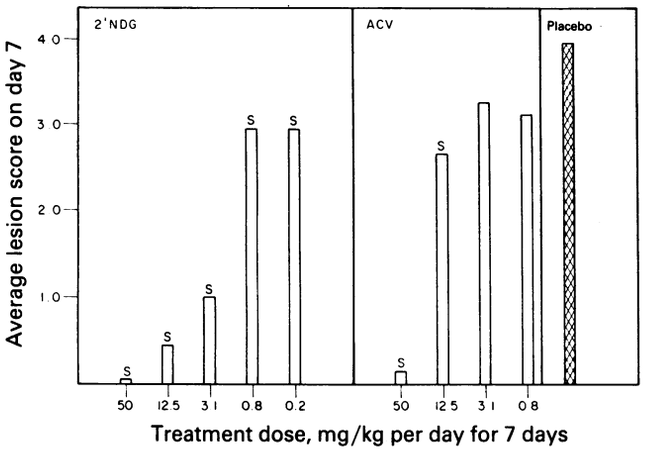

FIG. 3. Oral treatment of mice after orofacial infection with HSV-1. HRS/J mice (20 g) were infected on the snout with HSV-1 and then treated twice daily for 7 days with 2'NDG or ACV at the indicated dosage. Orofacial lesion severity was scored on day 7, with 0 for no herpetic lesions through 4 for 100% lesion development over the orofacial area. S, significantly different from placebo controls.

vaginitis, none of these animals showed progressive disease resulting in paralysis and death.

For systemic infection, vaginal infection, or orofacial infection, significant protection could be conferred even if oral treatment was delayed until 72 hr after infection (data not shown).

Biochemical Studies of 2'NDG. Kinetic studies of the phosphorylation of the three substrates deoxythymidine, ACV, and 2'NDG indicated that 2'NDG is a much better substrate for the viral TK than ACV is (Table 2). The V_{max} attained with 2'NDG is higher than that of deoxythymidine and is 4 times higher than that for ACV. The V_{max}/K_m ratio for 2'NDG is 30-fold higher than that for ACV.

An even greater difference in relative substrate efficiency was observed when the monophosphates of ACV and 2'NDG were compared as substrates for cellular GMP kinase (Table 3). 2'NDG-MP is an excellent substrate, comparable in its kinetic constants to dGMP, whereas ACV-MP is a very poor substrate, with a V_{max}/K_m ratio 1/492 of that for 2'NDG. The values for ACV-MP included in Table 3 are comparable to those reported by Miller and Miller (15).

It should be noted that neither ACV nor 2'NDG is an efficient substrate for the TK of uninfected HeLa cells. When compared at 100 mM, both antivirals were phosphorylated at rates lower than 1.5% of that of deoxythymidine.

Table 2. Comparison of the kinetic constants of deoxythymidine, ACV, and 2'NDG in the HSV-1-induced TK reaction

Substrate	K_m, μM	V_{max}, pmol/min	V_{max}/K_m
Deoxythymidine	8.5	218	25.7
ACV	426	61	0.14
2'NDG	66	280	4.25

The assay mixtures (100 μl) contained: 50 mM KPO_4 buffer, pH 6.5; 3 mM $MgCl_2$; 3 mM ATP; 100 μg of bovine serum albumin; HSV-1-induced TK; and various concentrations of the three substrates, [^{3}H]deoxythymidine, [^{14}C]ACV, and [^{14}C]2'NDG. Incubations were at 37°C for 10 min. After incubation, 80-μl aliquots were placed on DEAE paper disks (Whatman DE81, 2.5 cm), which were then washed successively with water, twice with 50% (vol/vol) ethanol containing either 0.5 mM deoxythymidine (when deoxythymidine was the substrate) or 0.5 mM guanosine (when the substrate was ACV or 2'NDG), and once with absolute ethanol; the discs were dried and their radioactivities were measured in Aquasol-2. Ten concentrations, each in duplicate, were used for each substrate. The values reported are the averages of three determinations. The data were computed by using an SAS (SAS Institute, Cary, NC) nonlinear regression program according to the equation $v = V_{max}[S]/([S] + K_m)$.

Table 3. Comparison of the kinetic constants of dGMP, ACV-MP, and 2'NDG-MP in the GMP kinase reaction

Substrate	K_m, μM	V_{max}, μmol/min per mg	V_{max}/K_m
dGMP	124	17.2	0.14
ACV-MP	316	0.20	0.0065
2'NDG-MP	22	7.1	0.32

Various amounts of the substrates were placed in the cuvette of a Cary recording spectrophotometer kept at 25°C with an assay mixture (400 μl final volume) containing 0.1 M Tris/acetate buffer at pH 7.6, 0.1 M KCl, 10 mM $MgCl_2$, 4 mM ATP, 1.5 mM phospho*enol*pyruvate, 100 μg of bovine serum albumin, 0.2 mM NADH, lactate dehydrogenase at 4 units/ml, and pyruvate kinase at 2 units/ml. The reaction was started with the addition of GMP kinase; the kinase was used at 0.35 unit/ml for ACV-MP and 0.008 unit/ml for dGMP and 2'NDG-MP. Ten different concentrations, each in duplicate, were used for each substrate. The initial velocities, calculated from the linear part of the absorbance curves, were used for the computation of the kinetic constants by the SAS program mentioned for Table 2.

The rates of triphosphate formation from 2'NDG and ACV were compared in crude extracts of HSV-1-infected HeLa cells, supplemented with purified viral TK and cellular GMP kinase (Fig. 4). Under these experimental conditions, 2'NDG was phosphorylated to the triphosphate at a much higher rate than was ACV.

The triphosphate of 2'NDG is a potent inhibitor of the HSV-1-induced DNA polymerase and to a lesser extent of the normal cellular DNA polymerases. Preliminary evidence obtained with crude preparations of the triphosphates and crude extracts of infected and uninfected HeLa cells as the sources of the enzymes indicated that ACV and 2'NDG triphosphates have comparable inhibitory activities (data not shown).

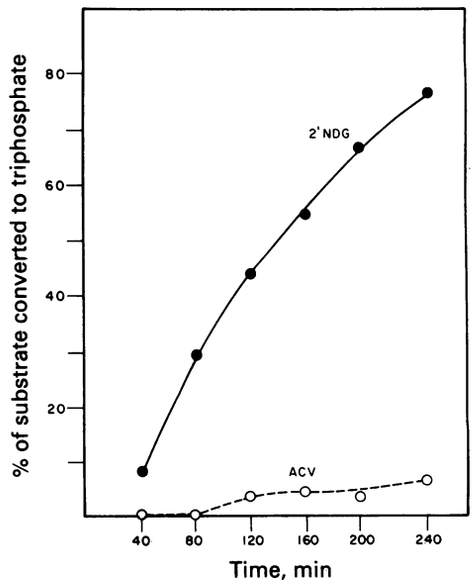

FIG. 4. Rates of triphosphate synthesis from ACV and 2'NDG. ACV or 2'NDG (40 μg) was incubated at 37°C in a 200-μl assay mixture containing 0.0014 unit of HSV-1 TK, 0.04 unit of GMP kinase, 12.5 units of creatine kinase, 20 μl of crude extract of HSV-1-infected HeLa cells, 7.5 mM phosphocreatine, 2.5 mM ATP, 2.5 mM $MgCl_2$, 2 mM dithiothreitol, 2.5 mM NaF, 50 mM Tris·HCl at pH 7.5, and bovine serum albumin at 0.5 mg/ml. Aliquots were removed at the times indicated and the amount of triphosphate formed was determined by HPLC.

DISCUSSION

2'-NDG is conveniently synthesized in a four-step sequence from 4-chloromethyldioxolane. Lewis acid-catalyzed ring opening of the dioxolane with acetic anhydride produced a mixture of isomers that was more easily resolved than the corresponding mixture of acyl triacetates (1) due to the presence of the chloro substituent. The acetolysis and acid-catalyzed fusion of the appropriate side-chain intermediate with diacetylguanine proceeded in good yield to furnish the protected acyclonucleoside with regioselectivity. Aqueous methylamine was employed in the near quantitative deprotection to afford 2'NDG.

2'NDG is an effective and selective inhibitor of herpes virus infection. Its antiherpetic activity correlates with the rapid phosphorylation to 2'NDG-MP by HSV TK. Its enhanced antiviral activity over ACV is presumably in part a result of its more rapid conversion to 2'NDG triphosphate, a potent inhibitor of the viral DNA polymerase.

However, for two herpes group viruses, HCMV and EBV, 2'NDG has substantially more antiviral activity than ACV *in vitro*. The reasons for this difference are not clear at present. Both of these viruses code for and require novel DNA polymerases for genome synthesis, and both induce a significant increase in TK activity upon infection (16–18). HCMV apparently does not code for its own TK and it is not clear whether the increased TK activity in EBV infection is virus encoded or cellular in origin (19, 20). It is possible that sufficient phosphorylation of 2'NDG occurs in infected cells and that the viral DNA polymerase has an increased sensitivity towards the 2'NDG triphosphate. Alternatively, 2'NDG may inhibit HCMV or EBV replication by another, unrelated, mechanism.

Oral treatment with 2'NDG was substantially more effective than that with ACV against both localized and systemic HSV-1 infections, as well as HSV-2 vaginal infections in mice. The potency of 2'NDG relative to ACV ranged from 6-fold to 50-fold, depending on the nature of the infection and treatment regimen. This is in agreement with the previous report (1) that, by subcutaneous injection, 2'NDG was significantly more effective than ACV in preventing encephalitis and death due to HSV-1 i.p. infection in mice. Taken together, these data suggest that under conditions of limited uptake and possible rapid clearance in mice as demonstrated for ACV (21), the more rapid conversion of 2'NDG to triphosphate results in enhanced antiviral efficacy.

At this very early juncture in the development of 2'NDG as an antiviral drug, it would appear to have great promise. At present, no effective therapy exists for HCMV infections. HCMV is a leading cause of disease in organ transplant recipients and effective antiviral therapy could substantially reduce the risk associated with transplant procedures (22, 23). 2'NDG must undergo extensive safety assessment and pharmacokinetic examinations. Clinical evaluation of 2'NDG will depend on the results of these studies.

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