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Comparative anti-herpesvirus activities of 9-(1,3-dihydroxy-2-propoxymethyl)guanine, acyclovir, and two 2′-fluoropyrimidine nucleosides

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Summary

9-(1,3-Dihydroxy-2-propoxymethyl)guanine (DHPG), was evaluated in cell culture and in animals for its inhibitory effect on herpes simplex viruses. Compounds run for comparison included acyclovir, 2′-fluoro-2′-deoxy-5-iodo-arabinofuranosylcytosine (FIAC), and 2′-fluoro-2′-deoxy-5-methyl-arabinofuranosyluracil (FMAU). In plaque reduction assays DHPG, acyclovir, FIAC, and FMAU were inhibitory to six herpes types 1 and 2 virus strains at concentrations of 0.2–2.4 μM. These concentrations were much lower than those required to inhibit Vero cell proliferation. In guinea pig vaginal infections, DHPG provided significantly greater inhibition of herpetic lesions than did acyclovir. In a herpes type 2 infection model in mice, DHPG, and FMAU were active at 5 mg/kg, whereas acyclovir and FIAC showed no statistically significant effect at 80 mg/kg. In a herpes type 1 encephalitis model, DHPG and FMAU were active at doses <10 mg/kg, with FMAU being about 4 times more potent than DHPG in that model.

DHPG; acyclovir; Ara-A; FIAC; FMAU; herpesvirus inhibition

Introduction

The purine nucleoside, 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) (also referred to as 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl} guanine and 2′-nor-2′-deoxyguanosine in the literature [1,17]) is a potent inhibitor of herpes simplex, cytomegalo-, Epstein-Barr and varicella-zoster virus replication [3,5,16,17]. It is one of a large number of compounds selectively phosphorylated by herpesvirus but not cellular thymidine kinase [2,5,16]. In animal studies DHPG has been shown to be effective against herpes types 1 and 2 encephalitis [5,6,13,16], herpes type 1 orofacial

disease [5], and herpes type 2 vaginitis [16]. In the encephalitis studies, the potency of DHPG exceeded that of acyclovir and FIAC (2'-fluoro-2'-deoxy-5-iodo-arabinofuranosylcytosine).

In this report we compared the antiviral activity of DHPG with that of other effective antiherpes agents. These nucleosides included acyclovir, FIAC, and FMAU (2'-fluoro-2'-deoxy-5-methyl-arabinofuranosyluracil) [9]. Each of the compounds selected for comparison with DHPG is inhibitory to herpes viruses in certain animal models. The analogs were tested for their effects in cell culture against several virus strains, for cytotoxicity, and for their effects on herpetic infections in mice and guinea pigs.

Materials and Methods

Antiviral agents

DHPG and acyclovir were synthesized by previously described procedures [13,14]. FIAC and FMAU were gifts of Jack J. Fox, Sloan Kettering Institute, New York. Compounds were stored in cell culture medium at -20°C as 2 mM solutions, or made up fresh daily in saline for animal studies.

Cells and viruses

African green monkey kidney (Vero) cells, obtained from the American Type Culture Collection, Rockville, MD, were used for plaque and cytotoxicity assays. Cells were passaged in Eagle's medium (EMEM) containing 10% fetal bovine serum, 10 mM Hepes buffer, and 0.12% $NaHCO_3$. The viruses used were herpes simplex type 1 (HSV-1), strains F, MGH 10 and Shealey; and herpes simplex type 2 (HSV-2), strains G, Lovelace and MS. The sources of procurement and methods of propagating these viruses are stated in an earlier publication [16].

Plaque and cytotoxicity assays

Plaque assays were performed in 6-well Costar brand (Bellco Glass Co., Vineland, NJ) plates using methylcellulose-containing medium overlays as described previously [16]. Plaques were counted after 4 days incubation at 37°C in a 5% CO_2 incubator. Cell proliferation assays were performed in 6-well plates allowing 1×10^4 cells in each well to replicate 4–6 days prior to determining cell numbers. At least 5 concentrations of drug were run for each of the above experiments. 50% inhibitory doses were determined using a semilog probit analysis [7] computer program.

Animal studies

Vaginal infections. Hartley strain guinea pigs, weighing 220–270 g, were purchased from Camm Research Institute, Wayne, NJ. Their vaginal tracts were swabbed with cotton-tipped applicators dipped in 0.1 N NaOH 1 h pre-infection. At infection 1×10^4 pfu of HSV-2 (G strain) in a 0.1 ml volume were inserted into each vagina using an oral gavage needle. Topical treatments were twice a day for 5 days starting at 24, 48, or 72 h after virus inoculation using a 25% propylene glycol base cream for drugs and placebo.

The concentration of DHPG or acyclovir in the cream was 5%. Lesion scores in the guinea pig model were quantified by the method of Kern et al. [12]. Lesion score differences between drug and placebo were evaluated by a two-tailed Mann–Whitney U-test.

Herpes type 2 encephalitis. Swiss-Webster mice (Simonson Labs, Gilroy, CA), weighing about 20 g each, were infected intraperitoneally with 5×10^4 pfu (about 10 LD_{50}) of HSV-2 (G strain). Compounds were administered subcutaneously once a day for 4 days starting 6 h post-infection.

Herpes type 1 intracerebral infection. 3-week-old female Swiss-Webster mice, weighing about 13 g each, were obtained from Simonsen Labs. They were anesthetized with 0.8 mg Nembutal (Abbott Laboratories, North Chicago, IL) administered intraperitoneally, and inoculated in the right cerebral hemisphere with 0.025 ml of medium containing 250 pfu of HSV-1 (Shealey strain). Compounds were administered subcutaneously twice a day for 5 days starting 24 h after virus inoculation.

For all of the above studies, there were at least 20 animals in each dosage group or placebo control. Deaths were recorded through 21 days post-infection. All compounds were well tolerated at the doses given.

Results

Plaque inhibition and cytotoxicity assays

Evaluation of DHPG, acyclovir, FIAC, and FMAU against 6 strains of HSV-1 and HSV-2 (Table 1) showed 50 percent inhibition of virus at low drug concentrations. The agents inhibited HSV-1 strains at 0.2–1.0 μM and HSV-2 strains at 0.3–2.4 μM. Against any particular virus, the difference in ID_{50} values between the most and least active antiviral nucleosides was no greater than 3-fold, indicating that all compounds had relatively the same degree of potency. Inhibition of uninfected Vero cells by the

TABLE 1

Comparative antiviral efficacies of DHPG, acyclovir, FIAC and FMAU in vitro

Virus or cell	50% Inhibitory dose[a] (μM)			
	DHPG	Acyclovir	FIAC	FMAU
HSV-1 (F strain)	0.2	0.5	0.3	0.3
HSV-1 (MGH 10 strain)	0.5	1.0	0.5	0.8
HSV-1 (Shealey strain)	0.3	0.7	0.5	0.9
HSV-2 (G strain)	0.3	0.5	0.9	0.8
HSV-2 (Lovelace)	0.4	0.8	0.9	0.5
HSV-2 (MS strain)	1.8	2.4	1.3	2.1
Vero cells	900	1400	100	40

[a] Dose causing a 50% reduction of HSV plaque forming units compared to drug-free infected Vero cell cultures, or of cell proliferation in uninfected cultures.

nucleosides was observed at concentrations much higher than those required to inhibit herpes viruses (Table 1). The 2'-fluoropyrimidine nucleosides were much more inhibitory to cell replication than were the acyclic nucleosides in the assay, with FMAU being the most potent anticellular agent.

Treatment of herpes type 2 vaginitis

Topical treatments with 5% DHPG or 5% acyclovir were conducted in guinea pigs (Fig. 1). DHPG treatments starting at 24 and 48 h were approximately equally effective in inhibiting lesion development compared to the control ($P < 0.02$). At 72 h DHPG therapy was also statistically active ($P < 0.05$). Acyclovir, however, was only effective when treatment started 24 h post-infection ($P < 0.05$) but not at 48 or 72 h.

Treatment of herpes type 2 encephalitis

In the initial study, DHPG was compared with acyclovir, FIAC, and FMAU to determine their protective effects against HSV-induced mortality (Table 2). Acyclovir and FIAC were not effective in preventing death at $\leqslant 80$ mg/kg but did increase mean survival times at the higher doses. In contrast, DHPG and FMAU prevented virus-induced mortality at 20 and 5 mg/kg, respectively. In a second experiment (Table 1 at bottom) where lower doses of the two active inhibitors were used, DHPG prevented death at 5 mg/kg, and FMAU was similarly effective at 2.5 mg/kg.

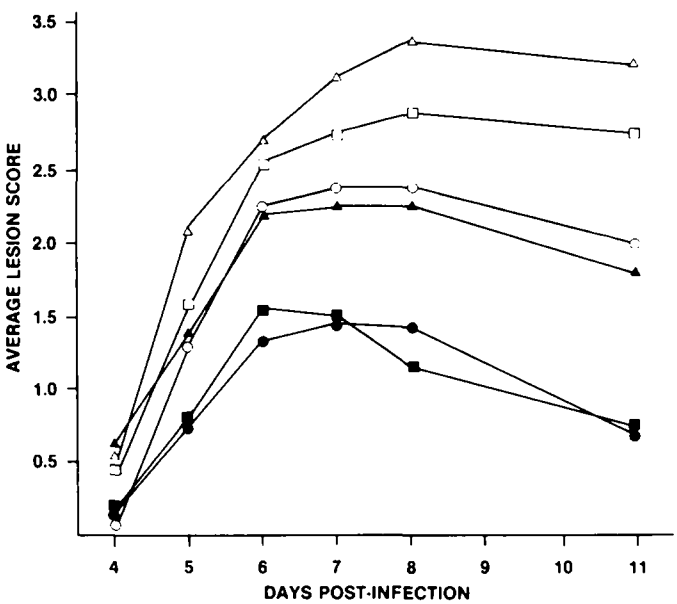

Fig. 1. The effects of DHPG and acyclovir on a herpes type 2 vaginal infection in guinea pigs. Topical treatments with 5% drug were twice a day for 5 days starting 24, 48, or 72 h after virus inoculation. ● = DHPG, 24 h; ■ = DHPG, 48 h; ▲ = DHPG, 72 h; ○ = acyclovir, 24 h; □ = acyclovir, 48 h; △ = placebo, 24 h.

TABLE 2

Effects of nucleoside analogs on HSV-2 induced mortality in mice

Compound	Dose (mg/kg per day)[a]	Survivors/ total	Survivor increase[b]	Mean survival time (days)[c]	Survival time increase[d]
Expt. 1					
Saline		2/20 (10)[e]		8.9 ± 2.1[f]	
DHPG	20	18/20 (90)	< 0.001	12.5 ± 0.7	< 0.05
	40	20/20 (100)	< 0.001	> 21	
	80	20/20 (100)	< 0.001	> 21	
Acyclovir	20	1/20 (5)	NS[g]	10.1 ± 3.0	NS
	40	3/20 (15)	NS	9.6 ± 1.2	NS
	80	4/20 (20)	NS	12.1 ± 1.9	< 0.001
FIAC	20	4/20 (20)	NS	9.9 ± 1.3	NS
	40	6/20 (30)	NS	11.9 ± 1.7	< 0.001
	80	4/20 (20)	NS	10.5 ± 1.0	< 0.01
FMAU	5	19/20 (95)	< 0.001	10.0 ± 0.0	NS
	10	18/20 (90)	< 0.001	10.5 ± 2.2	NS
	20	19/20 (95)	< 0.001	11.0 ± 0.0	NS
Expt. 2					
Saline		1/20 (5)		8.8 ± 2.3	
DHPG	5	8/20 (40)	< 0.02	11.7 ± 3.0	< 0.001
	10	14/20 (70)	< 0.001	12.3 ± 1.4	< 0.001
	20	14/20 (70)	< 0.001	12.7 ± 1.2	< 0.001
FMAU	1	1/20 (5)	NS	10.5 ± 2.2	< 0.02
	2.5	12/20 (60)	< 0.001	10.6 ± 1.5	NS
	5	18/20 (90)	< 0.001	12.0 ± 1.4	NS

[a] The dose was administered subcutaneously once a day for 4 days starting 6 h after inoculation.
[b] Probability value of statistical significance, determined by the two-tailed Fisher exact test.
[c] Of the mice that died.
[d] Probability value of statistical significance, determined by the two-tailed *t*-test.
[e] Percent survival.
[f] Standard deviation.
[g] Not statistically significant.

Treatment of a herpes type 1 intracerebral infection

Subcutaneous doses of DHPG, acyclovir and FMAU prevented death and increased mean survival time in mice inoculated intracerebrally with virus (Table 3). Acyclovir increased numbers of survivors only at 40 mg/kg, whereas DHPG and FMAU had activity at 5 and 2.5 mg/kg, respectively. Relatively speaking, the potency of FMAU appeared to be about 4-fold greater than DHPG in this model by comparing doses that gave approximately the same degree of protection.

TABLE 3

Effects of nucleoside analogs on an intracerebral HSV-1 infection

Compound	Dose (mg/kg per day)[a]	Survivors/ total	Survivor increase[b]	Mean survival time (days)[c]	Survival time increase[d]
Saline		1/20 (5)[e]		5.2 ± 1.1[f]	
DHPG	5	9/20 (45)	< 0.01	11.3 ± 3.9	< 0.001
	10	15/19 (79)	< 0.001	12.5 ± 2.9	< 0.001
	25	17/20 (85)	< 0.001	12.3 ± 3.2	< 0.001
Acyclovir	10	4/20 (20)	NS[g]	7.5 ± 1.6	< 0.001
	20	2/20 (10)	NS	7.0 ± 1.7	< 0.001
	40	7/19 (39)	< 0.05	7.5 ± 1.5	< 0.001
FMAU	2.5	13/20 (70)	< 0.001	10.1 ± 4.5	< 0.001
	5	16/19 (84)	< 0.001	7.3 ± 2.5	< 0.01
	10	18/20 (90)	< 0.001	8.5 ± 2.1	< 0.001

[a] Half daily doses were administered at 8 a.m. and 4 p.m. for 5 days starting 24 h after inoculation.
[b] Probability value of statistical significance, determined by the two-tailed Fisher exact test.
[c] Of mice that died.
[d] Probability value of statistical significance, determined by the two-tailed *t*-test.
[e] Percent survival.
[f] Standard deviation.
[g] Not statistically significant.

Discussion

The results of cell culture assays demonstrate that the four nucleosides have approximately the same degree of anti-herpesvirus activity in vitro but differing degrees of cytotoxicity. The cytotoxicity of DHPG was slightly greater than that of acyclovir as determined by cell proliferation assay, but was much less than that of the two fluoropyrimidine nucleosides. Others have reported greater toxicities for FIAC and FMAU [8,9], but since lower viral ID_{50} values were also reported, the differences in values may be due to assay conditions or the specific line of Vero cells used for testing. Although the in vitro results cannot be fully predictive of toxicity which will be encountered in vivo, these data suggest that the fluoropyrimidine nucleosides may potentially be more toxic in animals than the acyclic guanine nucleosides.

In the animal studies, DHPG was highly effective given orally, subcutaneously or topically in herpes encephalitis and vaginitis infections. Its superior potency relative to acyclovir and FIAC has already been documented in a herpes type 1 mouse model [5], but comparison to FMAU has not appeared in print prior to this study. It appears from these data that FMAU may be 2–4-fold more potent than DHPG in preventing death due to virus induced encephalitis, whether the virus is inoculated intraperitoneally or intracerebrally. We were unable to run FIAC and FMAU in all in vivo tests for comparison with DHPG due to the limited quantities of drug provided.

Although the relative potency of FIAC compared to the other nucleosides in treating a HSV-1 intracerebral infection was not determined here, a study of this type was recently reported [15]. It was evident from those data that FMAU showed the greatest degree of potency, and that FIAC was somewhat more protective than acyclovir. Another study compared DHPG, acyclovir and bromovinyldeoxyuridine (BVDU) in a HSV-1 encephalitis model where an intranasal route of virus inoculation was used [6]. There the protective activity of DHPG could be attributed to a marked reduction in recoverable virus in all parts of the brain, an effect not achieved with acyclovir or BVDU.

In the in vivo experiments therapy was continued for a fairly short time relative to the duration of the disease. By increasing the length of treatment, one would expect to see greater survivor increases in the mouse encephalitis studies. In the guinea pig vaginal model, peak virus shedding occurs on days 2–4 with little virus recovered in vaginal swabs after day 7 post-infection (unpublished data). Because virus replication precedes lesion development, there would not be a significant degree of difference in lesion scores if therapy were extended past 5–7 days. We have observed in other studies that the time of initiation of therapy has more impact on reducing the severity of the disease than the number of days that treatments are given. Treatments initiated earlier than 24 h are especially effective in inhibiting lesion development.

At the present time, the reasons for enhanced potencies of DHPG and FMAU are unclear. One might suspect that pharmacological factors would contribute to the drugs' reaching and persisting at the sites of infection for longer periods of time than do acyclovir and FIAC. Preliminary pharmacological tests performed in mice, dogs, and monkeys show that the metabolism and elimination of DHPG are very similar to acyclovir (unpublished data). These parameters, however, may not be true indicators of the intracellular pharmacology of the compounds. We have found that DHPG triphosphate, the antiviral metabolite of DHPG breaks down at a much slower rate than does acyclovir triphosphate when drug is removed from infected cultures (unpublished data). This could have profound implications on the ability of DHPG to be virus inhibitory in vivo after the decline of drug in the blood. It may be that FIAC is less active than FMAU because of its rapid metabolism to other species [11]. A recent discovery that FMAU is present at higher concentrations than FIAC in both blood and brain may also be a major factor in its superior potency [15].

Another aspect to potency may relate to the irreversibility of a compound against virus replication. Cheng and colleagues have shown that DHPG activity against herpes type 1 in vitro persists after drug is removed [3]. The activities of FMAU and FIAC are also irreversible (Y.-C. Cheng, personal communication). Analogous studies with acyclovir have not been conducted. The irreversibility phenomenon may be associated with incorporation of nucleoside internally into DNA, an event which cannot occur with acyclovir since it lacks the 3′ position [4], but has been demonstrated for DHPG [10] and the 2′-fluoropyrimidine nucleosides [11].

The data presented in this report illustrate that the relative in vivo potencies of structurally related nucleosides cannot be predicted merely on the basis of cell culture assays. For proper evaluation of compounds in vivo, animal tests should be performed judiciously, i.e. with differing treatment regimens, in different animal models, with

varying vehicles for topical treatment, etc. Otherwise, animal experiments may not allow any better predictions of a compound's potential than cell culture assays. In our other studies comparing DHPG activity to that of acyclovir, DHPG has always been the more active nucleoside although the difference in potency varied with each kind of test design, indicating that a particular regimen may be more favorable to one compound than another. At this stage it is not known whether the impressive antiviral activities of DHPG and FMAU are paralleled by increased toxic side effects for the animal.

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