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Efficacy of the Acyclic Nucleoside 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine Against Primary and Recrudescent Genital Herpes Simplex Virus Type 2 Infections in Guinea Pigs

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The acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) was evaluated for its efficacy in protecting guinea pigs from primary and recrudescent infections of herpes simplex virus type 2. Vaginally infected guinea pigs were treated twice a day with DHPG at 25 mg/kg per dose for 3 weeks. Subcutaneous doses were started 3 h, 24 h, or 5 weeks after virus inoculation. Treatment starting at 3 or 24 h reduced the severity of the primary infection by >70% when lesions were graded for 3 weeks; lesion duration was lessened by >55%. For 6 weeks after treatment, the number of recrudescent lesions was reduced by >60%, and the duration of the recrudescences declined by >40%. When dosing was started at 3 h postinfection, 33% of the animals did not develop any sign of primary or recrudescent infection throughout the 9-week test. By comparison, all the animals treated with DHPG starting at 24 h or with saline became infected. A 3-week DHPG regimen starting 5 weeks postinfection reduced the number of animals that developed recrudescent lesions by 70%. When treatment ended, however, recrudescent episodes in the animals increased to the level of saline-treated controls. These results suggest that (i) DHPG is highly effective in reducing the severity of both primary and recrudescent lesions of herpes simplex virus type 2, (ii) early treatment of a primary infection or treatment of recrudescences reduces the incidence of recrudescences, and (iii) the drug appears to have no effect on the latent form of the virus, as the incidence of recrudescences increases when DHPG treatment is ended.

Herpes simplex virus type 2 (HSV-2) typically causes an acute primary genital infection in humans which is followed by recurrent episodes of lesser severity. Studies of the pathogenesis of herpes simplex virus recurrent diseases in humans and animals indicate that cells in the sensory ganglia serve as a reservoir for the virus (2, 3, 19, 24, 27). The virus travels along nerves from the skin to establish itself in a latent, reactivable form in the ganglia (6, 14, 25). Whether the virus is present in a nonreplicating state or whether there is low but continuous gene expression during latency is not yet known (8, 17, 28).

Recently, an animal model has been developed for genital herpes in guinea pigs which resembles the human pathogenesis more closely than other models (10, 20, 23). Guinea pigs inoculated intravaginally with HSV-2 (MS strain) undergo a self-limiting primary infection which is characterized by vesiculo-ulcerative lesions on the external genital skin. As in humans, the animals, after recovering from the primary infection, develop recrudescent vesicular lesions and harbor latent HSV-2 in dorsal root ganglia. This model has been used to test the efficacy of the nucleoside 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine, acyclovir [ACV]) against primary and recurrent genital herpes (9; L. R. Stanberry, E. R. Kern, J. T. Richards, and J. C. Overall, Jr., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 419, 1982).

In the present study, we used this guinea pig model to examine the effect of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) on both primary and recurrent genital infections due to HSV-2. This compound is also known as BIOLF-62 (22), 2'NDG (1), and BW759 (18) depending on the source of the drug. DHPG has been reported to be active against several herpesviruses both in vitro and in vivo (1, 12, 21, 22) as well as against cytomegalovirus (5, 21) and Epstein-Barr virus (5) in vitro. During treatment, DHPG was evaluated for its efficacy against virus-induced primary and recrudescent

lesions. After treatment, the data obtained with recrudescences were used to postulate the effect of DHPG on the establishment of latency and on latent virus.

MATERIALS AND METHODS

Animals. Female Hartley strain guinea pigs (Charles River Breeding Laboratories, Wilmington, Mass.) (weight range, 195 to 245 g) were used for the infection. Animals were weighed at least once a week throughout the tests.

Virus and cells. HSV-2, MS strain, was obtained from Earl Kern, University of Utah School of Medicine, Salt Lake City, Utah. The virus was propagated in human laryngeal carcinoma (HEp-2) cells purchased from the American Type Culture Collection, Rockville, Md., and plaque was titrated in African green monkey kidney (Vero) cells.

Virus inoculation. The vagina of each guinea pig was swabbed with 0.1 N sodium hydroxide 30 min before infection. The animals were then inoculated intravaginally with 3.3×10^3 PFU of HSV-2 in Eagle minimum essential medium by infusing 0.1 ml with a ball tip needle and syringe. This method consistently produces a ≥90% rate of infection. The animals were randomized after inoculation. Details of the pathogenesis of the primary infection and a description of recurrent lesions are given by Stanberry et al. (23).

Treatment with antiviral compound. DHPG was synthesized as described previously (12). The dry powder was suspended fresh daily in physiological saline and sonicated for 10 min before administration. The solubility of DHPG at physiological temperature and pH was >5 mg/ml. Guinea pigs were injected subcutaneously twice a day for 3 weeks, starting 3 h, 24 h, or 5 weeks postinfection. The two daily injections were given 8 h apart at a dose of 25 mg/kg (50 mg/kg per day). This amount was adjusted as the animals gained weight. Control animals were treated with saline, starting 3 h or 5 weeks postinfection.

Grading of primary or recrudescent lesions. The severity of the virus-induced primary lesions of the external genital skin was scored by a single investigator on a daily basis. This grading was done blind with the investigator unaware of the different dosing schedules and treatment regimens being used. The following scale was used for the primary lesions: 0 = no visible lesions, 1 = redness or one or two lesions, 2 = several discrete lesions, 3 = lesions massed together, 4 = ulcerating lesions massed together. Primary lesions were scored for 3 weeks after inoculation of the virus.

Mean lesion scores were determined by using the number of guinea pigs in a group which developed primary lesions rather than the total number of animals per group. Thus, the calculated scores are a measure of the severity of the lesions themselves rather than a measure of lesion severity in the group as a whole. The inclusion of uninfected animals in these calculations would have favored DHPG treatment more than is shown in the present results, since fewer animals became infected in these groups.

Guinea pigs that had recovered completely from the primary infection, with no lesions for at least 2 days by the end of 3 or 4 weeks postinfection, were observed daily for recrudescences. The number of recrudescent lesions was recorded for each animal. The position of the lesion(s) was marked on a diagram. To calculate the number, frequency, or duration of recrudescences, a single episode was defined as the reappearance of the lesion(s) after two or more lesion-free days.

Recrudescences were monitored for 6 weeks after treatment of the primary infection starting 3 or 24 h postinfection. Those animals in each group which had cleared the primary infection by the end of 3 weeks postinfection were used for this analysis. Recrudescences were also monitored for 3 weeks during treatments starting 5 weeks postinfection and for 3 weeks after this treatment period. As was the case with mean lesion scores, the mean number of lesions was calculated by using the number of guinea pigs in a group which developed recrudescences rather than the total number of animals per group.

Statistical analyses. Statistical evaluation of differences in the number of animals which developed primary or recrudescent lesions was done by Fisher exact probability, using the two-tailed test (13). Differences in lesion scores, numbers, and duration or animal weights were evaluated by a two-tailed *t* test (4).

RESULTS

Effects of DHPG on primary lesions. Subcutaneous DHPG treatment, administered during the initial HSV-2 vaginal infection, resulted in a significant reduction in the numbers of guinea pigs with primary lesions (Table 1). This reduction was dependent on the time that treatment was started. Only half of the animals developed primary lesions when treatment was started 3 h postinfection ($P = 0.004$ when compared with saline-treated controls). However, the number of animals that developed lesions was ≥90% for DHPG treatment started 24 h postinfection or for saline-treated controls ($P > 1.0$). The average weights of treated and control animals (272 ± 20 and 280 ± 26 g, respectively) were not significantly different at the end of the 3-week treatment period ($P > 1.0$).

The primary infection cleared more quickly with DHPG treatment than with saline-treated controls (Fig. 1). At day 21, for example, lesions had been absent for 7 days with DHPG treatment started 3 h postinfection. At this time, only 20% of the animals had lesions with DHPG treatment started at 24 h. With saline treatment, 60% still had a visible infection.

In addition, the severity of the primary lesions, as measured by mean lesion score and duration, was lessened with DHPG treatment. The period of greatest severity ranged from days 4 to 10 for DHPG at 3 h, days 4 to 13 for DHPG at 24 h, and days 4 to >21 for saline treatment (Fig 1). For both treatments, the duration of the primary lesions was shortened by >55% ($P < 0.001$), and mean lesion scores were reduced by >70% ($P < 0.0001$) when compared with saline-treated controls (Table 1).

Effects of DHPG on recrudescent lesions when administered during primary infection. DHPG

TABLE 1. Development of HSV-2 primary or recrudescent lesions in vaginally infected guinea pigs during or after treatment with DHPG started at 3 or 24 h for the primary infection or at 5 weeks for the recrudescent infection

Treatment with DHPG[a]		Disease status									
		During treatment period						Posttreatment period			
				Primary episode		Recrudescent episode				Recrudescent episode	
Time initiated[b] and control	Duration (wk)	Observation interval (wk)[b]	No. of animals with lesions/total	Lesion score[c]	Duration (days)[c]	No. of lesions[c]	Duration (days)[c]	Observation interval (wk)[b]	No. of animals with lesions/total	No. of lesions[c]	Duration (days)[c]
3 h	1–3	1–3	13/25[d]	0.23[d]	3.0[d]	NA[e]	NA	4–9	16/24[d]	0.43[d]	2.8[d]
24 h	1–3	1–3	23/25	0.55[d]	5.7	NA	NA	4–9	19/19	0.57[d]	2.9[d]
Saline	1–3	1–3	52/58	2.02	13.7	NA	NA	4–9	26/26	1.58	4.9
5 w	5–7	5–7	3/15[d]	NA	NA	0.20	2.5	8–11	11/15	0.19	2.0
Saline	5–7	5–7	10/15	NA	NA	0.29	3.5	8–11	9/15	0.25	2.8

[a] DHPG administered subcutaneously twice a day at 25 mg/kg per dose.
[b] After virus inoculation.
[c] Mean; values for lesion score or number of lesions were determined by using the number of guinea pigs that developed either a primary or a recrudescent episode during the time interval studied rather than the total number of animals per group.
[d] Significantly different ($P < 0.05$) from saline-treated controls.
[e] NA, Not applicable.

treatment administered during the primary infection reduced the subsequent HSV-2 recrudescent lesions. As was the case with the primary lesions, the reduction was dependent on the time that treatment was initiated. When treatment was started 3 h postinfection, only 67% of the animals developed recrudescences for 6 weeks posttreatment (Table 1). However, all the animals first treated with DHPG at 24 h developed recrudescences, as did those with saline treatment. In addition, animals first treated at 3 h were more likely to develop only one recrudescent episode compared with the other two groups (Table 2).

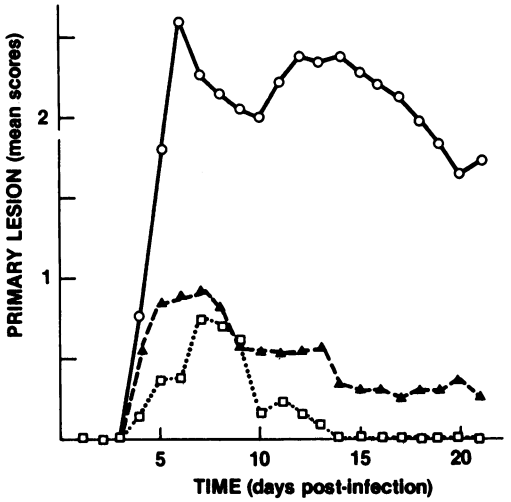

FIG. 1. Daily mean score of HSV-2 primary lesions in vaginally infected guinea pigs during 3 weeks of treatment with DHPG, starting 3 h (□; 25 animals) or 24 h (▲; 25 animals) postinfection, or with saline (○; 58 animals). Lesions were scored by using a range of 0 to 4 with 0 = none, 1 = redness or one to two lesions, 2 = several discrete lesions, 3 = lesions massed together, and 4 = ulcerating lesions massed together. Mean scores were determined by using the number of guinea pigs that developed a primary lesion rather than the total number of animals per group. P values for either of the DHPG-treated groups were <0.0001 when compared with the saline-treated control over the 3-week treatment period.

Another effect of DHPG treatment was to lessen the severity of the recrudescent episodes, as measured by mean lesion number and duration. There was >60% reduction in the number of lesions and >40% reduction in the duration of each episode when analyzed for 6 weeks after either treatment (Table 1).

Effect of DHPG on recrudescent lesions. DHPG treatment administered during the recrudescent phase to animals which had developed and cleared a primary infection significantly reduced the number of episodes. When initiated 5 weeks postinfection, 80% of the animals did not develop a single recrudescence during the 3-week period of treatment, whereas 67% of the saline-treated animals did (Table 1; $P = 0.025$). However, the number of lesions and the duration of each episode, for the few animals experiencing recrudescences, were not statistically different from those of the saline-treated controls ($P > 0.1$). The frequency of recrudescent episodes was also unchanged (Table 2).

When treatment ended, the number of animals with recrudescent episodes increased to that of saline-treated controls over a 3-week period

TABLE 2. Frequency of HSV-2 recrudescent episodes in vaginally infected guinea pigs during or after treatment with DHPG started at 3 or 24 h for the primary infection or at 5 weeks for the recrudescent infection

Expt no.	DHPG treatment[a]		Time of analysis (wk)	Animals with lesions/total	Recrudescent pattern (%)[b]					Mean no. of recrudescences/animals with lesions
	Start time and control	Duration (wk)			1×	2×	3×	4×	5×	
1	3 h	1–3	4–6	12/24	67	17	16	0	0	1.5
	24 h	1–3	4–6	16/19	38	38	19	5	0	1.9
	Saline	1–3	4–6	25/26	32	16	24	28	0	2.5
	3 h	1–3	7–9	12/24	50	34	8	0	8	1.8
	24 h	1–3	7–9	15/18	13	33	47	7	0	2.5
	Saline	1–3	7–9	26/26	15	54	27	4	0	2.2
2[c]	5 wk	5–7	5–7	3/15	33	67	0	0	0	1.7
	Saline	5–7	5–7	10/15	60	20	20	0	0	1.6
	5 wk	5–7	8–11	11/15	64	27	9	0	0	1.5
	Saline	5–7	8–11	9/15	56	22	22	0	0	1.7

[a] DHPG administered subcutaneously twice a day at 25 mg/kg per dose during these times.
[b] Percent animals with 1, 2, 3, 4, or 5 recrudescences. Percent was determined by using the number of guinea pigs which developed recrudescences over the time interval studied rather than the total number of animals per group.
[c] These guinea pigs had all developed and cleared a primary infection.

(Table 1). The number of animals developing lesions was >60% for either DHPG or saline treatment. The number of lesions and the duration of recrudescent episodes were also the same in both groups ($P > 0.1$). Likewise, the frequency of recrudescences was unchanged (Table 2).

DISCUSSION

The results presented here show that DHPG is highly effective in preventing or reducing the severity of both primary and recrudescent lesions of HSV-2. The lesions of a primary infection were less severe when graded and cleared more quickly with treatment. If treatment was begun early enough (3 h postinfection), the primary disease was prevented in a significant number of animals. In addition, recrudescent episodes were prevented in a significant number of animals when the compound was given prophylactically after an untreated primary infection. Thus, DHPG appears useful in suppressing the active virus of both primary and recrudescent infections.

It is postulated from the present results that early DHPG treatment of the primary infection may reduce the incidence of recrudescences because of a drug-related reduction in viral replication before the virus reaches the ganglia. Viruses are known to localize in ganglia starting at 20 to 24 h after inoculation (6, 19). The fact that the number of animals which developed recrudescences was reduced after treatment of the primary infection starting 3 h postinfection, but not at 24 h, suggests that DHPG at 3 h effectively blocked this spread. In addition, although DHPG was unable to prevent the establishment of latency at 24 h, it is possible that the reduction in severity of recrudescences occurred because some virions were prevented from reaching the ganglia. Like DHPG at 3 h, it did reduce the number of lesions and the duration of episodes for at least 6 weeks posttreatment. Other researchers have used these explanations in evaluating similar effects produced by different antiviral compounds (8).

Finally, the present results with DHPG suggest that the compound may have no effect on the latent form of the virus once it becomes established. When treatment was initiated 5 weeks postinfection, a time at which the latent virus is firmly entrenched, recrudescent episodes could be controlled only with continual DHPG therapy. The fact that the number of animals with recrudescences increased to the level of saline controls once the DHPG treatment was ended suggests that the drug is unable to destroy latent virus.

The observation that latent HSV-2 does not respond to systemic DHPG treatment is consistent with the mode of action of DHPG. The compound acts upon the replication of the virus rather than the virus itself. The mode of action of the drug requires its initial phosphorylation by virus-induced thymidine kinase leading to the eventual inhibition of viral DNA polymerase activity (1, 5, 21). If the viral genome is in a resting state, these enzymes necessary for virus replication would not be expressed. Thus, systemic treatment with DHPG would not eradicate the infection. This explanation has been proposed for the lack of efficacy seen with other antiviral compounds which are known to affect virus replication (8, 26).

The results obtained with DHPG are similar to those obtained for ACV. First, ACV, like

DHPG, was found to reduce the development and severity of a primary genital herpes infection in guinea pigs (9, 16). Second, during treatment with either compound, the mean number and duration of recrudescent episodes of genital herpes was reduced. After therapy, the number of episodes increased to the level of the control group (22nd ICAAC, abstr. no. 419). Finally, with DHPG, the incidence of recrudescences was reduced when treatment was started at 3 but not 24 h postinfection, whereas treatment of a primary infection with ACV in a number of different animal models reduced the establishment of latency (as measured by virus titers in ganglia) if initiated at 3 but not 24 h postinfection or later (7, 11, 15). However, since DHPG and ACV were not tested together in our experiment, the comparative efficacy of the two compounds against genital herpes remains unknown.

In summary, the present results indicate that (i) DHPG is highly effective in reducing the severity of both primary and recrudescent lesions of HSV-2, (ii) early treatment of the primary infection by DHPG or treatment of recrudescences reduces the incidence of recrudescences, and (iii) the drug appears to have no effect on the latent form of the virus, a fact which is consistent with its mode of action.

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