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Enhanced Efficacy of the Acyclic Nucleoside 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine in Combination with Alpha-Interferon Against Herpes Simplex Virus Type 2 in Mice

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The acyclic nucleoside DHPG [9-(1,3-dihydroxy-2-propoxymethyl)guanine] and recombinant human alpha-interferon of clones A/D potentiate each other's antiviral activity against a systemic infection with herpes simplex virus type 2. The effective dose at which 50% of the mice survived was lowered ~10-fold for DHPG when it was given in combination with a marginally effective dose of alpha-interferon and >10-fold for alpha-interferon when it was given in combination with a nontherapeutic dose of DHPG.

The combination of the acyclic nucleoside DHPG [9-(1,3-dihydroxy-2-propoxymethyl)guanine] with alpha-, beta-, or gamma-interferon (IFN) has been found to be synergistic against herpes simplex virus type 1 (HSV-1) and HSV-2 in vitro (1a; D. M. Moran, J. C. Overall, Jr., and E. R. Kern, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 749, 1983). Another analog, acyclovir (ACV) [9-(2-hydroxyethoxymethyl)guanine], has also been shown to be additive to synergistic with IFN-α or IFN-β against HSV-1 and HSV-2 (4, 7, 10; D. M. Moran et al., 23rd ICAAC, abstr. no. 749). In addition, a synergistic interaction between DHPG and either mouse IFN-β (MuIFN-β) (3) or MuIFN-γ (E. B. Fraser-Smith, D. A. Eppstein, Y. V. Marsh, and T. R. Matthews, Antiviral Res., in press) was recently demonstrated in vivo as measured by survival of mice infected with HSV-2.

We now report the results of an in vivo study in which the antiviral effect of DHPG in combination with human IFN-α was examined against the same HSV-2 infection in mice.

Female Swiss Webster mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass. weighing 14 to 17 g each were inoculated intraperitoneally (i.p.) with 10^3 PFU of HSV-2 strain G (American Type Culture Collection) and randomized into 15 groups of 20 mice each. This HSV-2 challenge consistently produced 100% mortality in saline-treated control mice.

Beginning at 24 h postinfection, 13 groups of mice were treated with different doses of recombinant human IFN-α-AD (rHuIFN-α-AD) injected i.p. or DHPG injected subcutaneously or both. The compounds were given within 1 h of each other once a day for 5 days. The remaining two groups of mice served as controls and were treated with either saline (DHPG control) or saline with mouse serum albumin (MSA; 0.1 mg/ml) (rHuIFN-αAD control). The mice were observed for mortality for 21 days after challenge. At the end of this period, all surviving mice were healthy.

DHPG (Syntex Research) and rHuIFN-α-AD (Hoffmann-La Roche, Inc.) were prepared as described previously (3). The specific activity of the rHuIFN-α-AD was 10^8 international reference units (IU) per mg as determined by titration on human GM2767 cells and standardization against National Institutes of Health HuIFN-α reference no. G-023-901-527 as described previously (3). This rHuIFN-α-AD hybrid cross-reacts very well on murine cells (6, 9) as well as in mice (5).

Table 1 shows the antiviral effect of a marginally effective dose of rHuIFN-α-AD in combination with various doses of DHPG. When given alone, rHuIFN-α-AD at 10^4 IU per mouse had little protective activity against HSV-2 infection. Only 15% of the IFN-treated mice survived, compared with no survivors for the saline-MSA-treated controls ($P > 0.1$, two-tailed Fisher probability test [8]). Likewise, DHPG alone had little protective activity at the two lowest doses used (≤20% survivors, $P = 0.09$ compared with the saline control). However, all doses of DHPG gave good protection against HSV-2 when given in combination with the marginally effective dose of rHuIFN-α-AD ($P < 0.002$).

Table 1 also shows the antiviral effect of a marginally effective dose of DHPG (1 mg/kg) in combination with various doses of rHuIFN-α-AD. With this dose of DHPG alone, 20% of the mice survived ($P = 0.09$ compared with the saline-treated control [no survivors]). Likewise, rHuIFN-α-AD by itself had little anti-HSV-2 activity at any of the concentrations used ($P > 0.1$ compared with the saline-MSA control). However, all doses of rHuIFN-α-AD gave good protection against HSV-2 when combined with the marginally effective dose (1 mg/kg) of DHPG ($P < 0.002$).

A synergistic interaction between these two drugs was confirmed by first determining the effective dose at which 50% of the mice survived (ED_{50}) (Table 1) using probit analysis (2) and then calculating the fractional protective dose index with the formula (ED_{50} of DHPG in combination)/(ED_{50} of DHPG alone) plus (ED_{50} of IFN in combination)/(ED_{50} of IFN alone) (1). The resulting index was <0.2; a value of ≤0.5 indicates potentiation.

The present in vivo results, which demonstrate a 10-fold enhancement of anti-HSV-2 activity with the combination of DHPG and rHuIFN-α-AD, are similar to previous in vivo results with DHPG and MuIFN-β (3). Because the same specific antiviral activity of IFN (10^4 IU/mouse) was found to reduce the ED_{50} of DHPG ~10-fold, natural MuIFN-β and rHuIFN-α appear to be equally active and may have similar modes of action against HSV. The differences in the

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TABLE 1. Survival of mice treated with different doses of DHPG or rHuIFN-α-AD or both against HSV-2 infection[a]

Treatment regimen		Response to treatment			ED_{50} alone or in combination	
DHPG (mg/kg)	rHuIFN-α-AD (IU/mouse)	No. of survivors/ total	Time to death (days)		DHPG (mg/kg)	rHuIFN-α-AD (IU/mouse)
			Median	Range		
Saline control		0/21	8	7–11		
0.3	0	3/20	9	8–12		
1	0	4/20	10	8–14	4	
3	0	10/20[b]	12.5	9–19		
18	0	16/20[b]	14	11–16		
0.3	10^4	11/20[b]	16	12–20		
1	10^4	13/20[b]	16	11–19	0.3	10^4
3	10^4	20/21[b]	16			
	Saline-MSA control	0/20	8	6–12		
0	3×10^3	1/20	9	7–16		
0	10^4	3/20	10	7–18		$>3 \times 10^4$
0	3×10^4	2/20	11	7–20		
1	3×10^3	15/20[b]	13	10–19		
1	10^4	20/20[b]			1	$<3 \times 10^3$
1	3×10^4	18/20[b]	11.5	11–12		

[a] Mice were treated with various concentrations of DHPG or IFN to determine the ED_{50} alone and with the marginally effective dose of each agent (DHPG, 1 mg/kg; IFN, 10^4 IU per mouse) combined with various concentrations of the other to determine the ED_{50} in combination.
[b] Significantly different ($P < 0.05$) from saline- or saline-MSA-treated controls.

actual ED_{50}s for DHPG between these two tests may be attributed to the flatness of the dose-response curves common to these studies. In addition, both IFN-α-AD and IFN-β alone showed little protective activity at doses up to 3×10^4 IU per mouse. On the other hand, IFN-γ was active even at a 10-fold-lower dose in separate tests (Fraser-Smith et al., Antiviral Res., in press).

Our in vivo results are also consistent with those of previous in vitro tests which showed synergistic antiviral activity with DHPG in combination with either IFN-α. In addition, in these in vitro studies, DHPG combined with either IFN-α or IFN-β potentiated activity against HSV-2 to the same degree. By comparison, DHPG combined with IFN-γ had >10-fold less synergistic activity in vitro (1a).

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