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Enhanced efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine in combination with gamma interferon against herpes simplex virus type 2 in mice

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Summary

The acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) and recombinant mouse interferon gamma (rMuIFN-γ) were evaluated for their efficacy alone and in combination against a herpes simplex virus type 2 systemic infection in mice. Intraperitoneally infected animals were treated once a day with the drugs at various concentrations for 5 days starting 24 h after inoculation. DHPG was given subcutaneously and rMuIFN-γ intraperitoneally. For DHPG, the effective dose at which 50% of the mice survived (ED_{50}) was lowered ≈10-fold from 3.4 to 0.25 mg/kg when given in combination with an ineffective dose of rMuIFN-γ (10^3 units per mouse). For rMuIFN-γ, the ED_{50} was lowered >10-fold from 6×10^3 to $<3 \times 10^2$ units per mouse when given in combination with a marginally effective dose of DHPG (1 mg/kg). Construction of an isobologram and calculation of the corresponding fractional protective dose index (<0.12 where values ≤0.5 are considered synergistic) indicates an enhanced protective interaction by the combination of the two drugs.

9-(1,3-dihydroxy-2-propoxymethyl)guanine; DHPG; interferon-γ; synergy; herpes simplex virus

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Introduction

The development of compounds with specific antiviral activity is a recent scientific advance. One of the most promising agents to date is the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG). This compound is structurally related to 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV). DHPG is also known as BIOLF-62 [1], 2'NDG [2], or BW759 [3]. DHPG has good protective activity against both systemic and local skin or genital infections caused by herpes simplex virus (HSV) [2,4–6].

Interferons (IFNs) are proteins with antiviral and immune regulatory activities. These agents are classified into 3 groups based on molecular and biological differences: α (leukocyte), β (fibroblast), and γ (immune). In recent animal studies, efficacy against HSV has been reported with IFN for both systemic and local infections of the eye, skin, and genital tract [7,8] (J.C. Overall Jr., T.J. Yeh and E.R. Kern, paper presented at Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, NV, 1983 program abstr. No. 392). In addition, in human clinical trials, both local and systemic infections of HSV have also benefited from IFN treatments [9–11]. To date, only IFN-α or -β have been used in these in vivo tests; sufficient quantities of IFN-γ have not been readily available.

Recently, combinations of human (Hu) IFN-α, -β or -γ, and either DHPG or ACV, have been found to be additive to synergistic against HSV grown in vitro [12–15] (D.M. Moran, J.C. Overall Jr. and E.R. Kern, paper presented at Intersci. Conf. Antimicrob. Agents Chemother. 23, Las Vegas, NV, 1983, program abstract No. 749). Moreover, our laboratory found that in vitro combinations of DHPG and HuIFN-α or -β were significantly better than with DHPG and HuIFN-γ [15]. We also found an enhanced efficacy of DHPG against an acute systemic HSV-2 infection of mice in combination with natural mouse IFN-β [16] or with recombinant human IFN-α (Fraser-Smith, E.B., Epstein, D.A., Marsh, Y.V. and Matthews, T.R., Antimicrob. Agents Chemother., (1985) in press). These findings led us to investigate the antiviral potency of DHPG in combination with mouse IFN-γ in vivo. We postulated that the in vitro and in vivo results with this combination might differ, since IFN-γ has unique immunomodulatory properties in the host, including augmentation of macrophage-cytotoxic [17] and natural killer cell [18] activities. Furthermore, for the first time, recombinant murine IFN-γ was available in sufficient quantity for such large-scale testing because of recent genetic engineering advances [19].

Materials and Methods

Female Swiss Webster mice (Charles River, MA; 14–17 g weight range) were inoculated intraperitoneally (i.p.) with 10^3 PFU of HSV-2 strain G (American Type Culture Collection). The animals were randomized into 14 groups of 20 mice each. This HSV-2 challenge was approximately 4 times the 50% lethal dose and consistently produced 100% mortality in saline-treated controls. In this model, death results from encephalitis after an initial multiplication of virus in several visceral organs [20].

Starting 24 h post-infection, 12 of these groups of infected mice were treated with different doses of rMuIFN-γ and/or DHPG. DHPG (Syntex) was solubilized daily in physiological saline before administration. The rMuIFN-γ (Genentech, Lot No. 1804/11) was diluted daily in phosphate-buffered saline with 0.1 mg/ml mouse serum albumin (MSA, Sigma). The rMuIFN-γ was injected i.p. and the DHPG subcutaneously. Both compounds were given within 1 h of each other once a day for 5 days. The remaining 2 groups of mice served as controls and were treated with either saline (DHPG control) or saline with MSA (rMuIFN-γ control). The mice were observed for mortality for 21 days following challenge. At the end of this time, all surviving mice were healthy.

The specific antiviral activity of rMuIFN-γ (5×10^6 units/mg) was assayed on L_{929} cells using a microtiter inhibition of cytopathic effect method with encephalomyocarditis virus as challenge [21]. Samples were standardized with the National Institute of Health reference reagent of mouse fibroblast interferon G002-094-511. The values obtained showed good agreement with those from Genentech.

Statistical evaluation of differences in the number of animals which survived the infection was done by a 2-tailed Fisher exact probability test [22]. Probit analysis [23] was used to calculate the effective dose of rMuIFN-γ or DHPG at which 50% of the mice survived (ED_{50}).

The possibility of a synergistic interaction between drugs was analyzed by constructing an isobologram and calculating the corresponding fractional protective dose index (FPDI) [24]. The FPDI was calculated as (ED_{50} of DHPG in combination/ED_{50} of DHPG alone) plus (ED_{50} of IFN in combination/ED_{50} of IFN alone). In the present study, values of <0.5 were considered to be synergistic, values between 0.5 and 1.5 were considered additive, and values of >1.5 were considered antagonistic.

Results

Fig. 1 shows the antiviral effect of a non-therapeutic concentration of rMuIFN-γ in combination with various doses of DHPG. rMuIFN-γ at 10^3 units (U) per mouse had no significant protective activity against this infection. The number of survivors was only 15% as compared to no survivors for the saline with MSA-treated controls ($P > 0.1$). Likewise, DHPG alone had little anti-HSV-2 activity at the 2 lower concentrations used ($P \geqslant 0.05$ compared to the saline control). However, all dose levels of DHPG gave good protection against HSV-2 when combined with the ineffective dose of rMuIFN-γ ($P < 0.002$).

Conversely, Fig. 2 shows the antiviral effect of a non-therapeutic concentration of DHPG in combination with various doses of rMuIFN-γ. DHPG alone at 1 mg/kg was only marginally effective against HSV-2. The number of survivors was 25% ($P = 0.05$ when compared to the saline-treated control with no survivors). Likewise, rMuIFN-γ by itself had no anti-HSV-2 activity at the two lower concentrations used ($P > 0.1$ compared to the MSA in saline control). At the highest concentration, rMuIFN-γ had some anti-HSV-2 activity ($P = 0.003$). However, all dose levels of rMuIFN-γ gave good protection against HSV-2 when combined with the marginally effective dose of DHPG ($P < 0.002$).

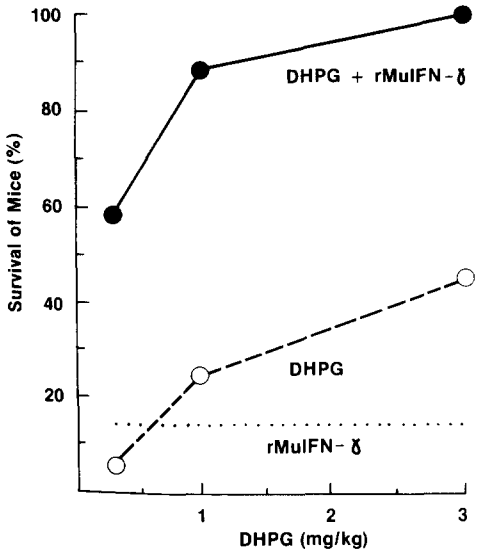

Fig. 1. Survival of mice treated with different doses of DHPG alone or in combination with an ineffective concentration of rMuIFN-γ (10^3 U/mouse) against an intraperitoneal HSV-2 infection. The dotted line indicates the survival level with rMuIFN-γ alone at 10^3 U/mouse.

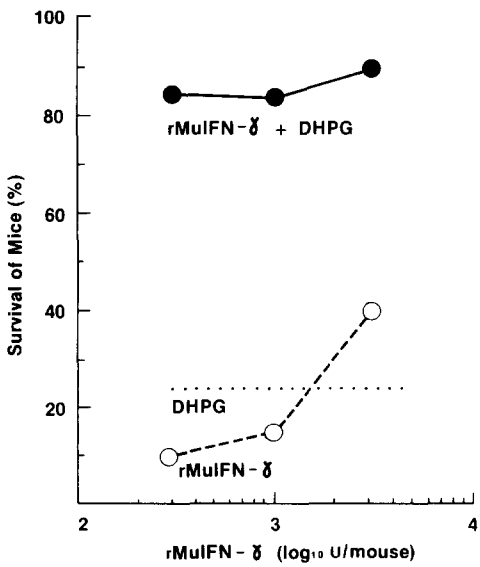

Fig. 2. Survival of mice treated with different doses of rMuIFN-γ alone or in combination with a marginally effective concentration of DHPG (1 mg/kg) against an intraperitoneal HSV-2 infection. The dotted line indicates the survival level with DHPG alone at 1 mg/kg.

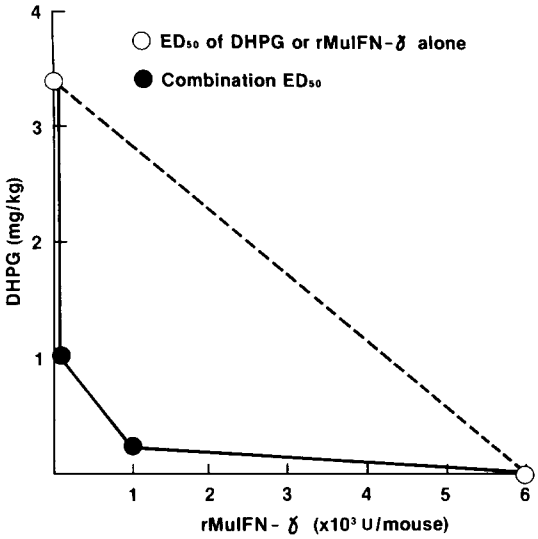

Fig. 3. Isobologram showing the effective dose of DHPG alone or rMuIFN-γ alone (○) or DHPG and rMuIFN-γ in combination (●) which produced a 50% survival of mice (ED_{50}) challenged with HSV-2. The dashed straight line represents the dose combinations that would be needed to produce an ED_{50} if the interaction of the two drugs was additive. The solid line represents the actual dose combinations found to produce an ED_{50} and indicates a synergistic interaction between the 2 drugs.

Lastly, an isobologram was drawn to compare the in vivo activity of the rMuIFN-γ/DHPG combinations against HSV-2 in mice. In Fig. 3, the dose of each drug that alone or in combination produced a 50% survival of mice challenged with HSV-2 (ED_{50}) was plotted on an arithmetic scale. The ED_{50} for DHPG alone was calculated to be $\approx$3.4 mg/kg, and for rMuIFN-γ alone $\approx 6 \times 10^3$ U/mouse. The dashed straight line connecting these two values represents the combination doses of rMuIFN-γ or DHPG necessary to yield an ED_{50} if the interaction of the 2 drugs were additive. The 2 data points below this line represent the actual dose combinations found to achieve an ED_{50}. The ED_{50} for DHPG in combination with rMuIFN-γ at 10^3 U/mouse was $\approx$0.25 mg/kg. The ED_{50} for rMuIFN-γ in combination with DHPG at 1 mg/kg was $< 3 \times 10^2$ U/mouse. If DHPG and rMuIFN-γ had acted additively, the curve for these points would lie closer to the straight line than to the origin. The fact that the curve lies closer to the origin indicates that there is a synergistic interaction between the two drugs. In addition, calculation of the corresponding FPDI gave a value of < 0.12 where values ≤ 0.5 are considered synergistic. This test was conducted three times with similar results.

Discussion

The present in vivo results, which demonstrate an excellent enhancement of anti-

HSV-2 activity with the combination of DHPG and IFN-γ, are in contrast to previous in vitro results which showed little potentiation of activity against HSV-2 with this same combination of drugs [15]. In addition, IFN-γ alone in the present study had significant antiviral activity at 3×10^3 U/mouse, whereas in a previous in vivo test IFN-β alone did not show protective activity even at 3×10^4 U/mouse [16]. These data are also in contrast to the in vitro tests, in which the antiviral activity of IFN-γ alone was significantly less than that of IFN-β by itself [15].

The facts that (a) the antiviral effect of IFN-γ either alone or in combination with DHPG is significantly less in vitro than it is in vivo, and (b) there are marked differences when IFN-γ is compared with IFN-β, suggest that the IFN-γ species has more than one mode of action. If the action of IFN-γ was mainly due to the establishment of an antiviral state in the target cell, a correlation between the in vitro and in vivo data could be expected. Thus, in addition to a direct interaction within the infected cell, the antiviral activity of IFN-γ may also be related to immunological events in the host. Recently, various researchers have found immunological differences between IFN-γ and IFN-α or -β relating to macrophage cytotoxicity [17] and augmentation of natural killer cell [18] activities. Future studies will examine this immunological modulation by IFN-γ as it relates to in vivo anti-HSV-2 efficacy.

To our knowledge, the present results represent the first time that IFN-γ has been shown to have in vivo antiherpetic efficacy. In addition, these data also represent the first time that potentiation of the antiviral activity of an acyclic nucleoside by IFN-γ has been demonstrated in vivo. The only other report of in vivo synergism between an acyclic nucleoside and any interferon species is our recent study with combinations of DHPG and IFN-β [16]. In a recent animal study, Rose et al. [25] tested ACV in combination with murine fibroblast IFN against murine cytomegalovirus (CMV) pneumonia. In these experiments, the combination of ACV plus IFN offered no increased antiviral activity compared to ACV alone.

In recent clinical trials, Colin et al. [26] and De Koning et al. [27] tested ACV in combination with human leukocyte IFN against herpes keratitis. An increased antiviral activity was found with the combination. However, as no data were presented on the effect of the IFN-α alone on the course of the disease, it was not possible to determine whether the combination was only additive or actually synergistic. More recently, Wade et al. [28] treated CMV pneumonia in humans with high-dose acyclovir and human leukocyte IFN. As in the animal study, this combination was ineffective against CMV.

Perhaps a protective effect against CMV in vivo could be obtained by a combination of DHPG and IFN, since human CMV is more sensitive to DHPG than to ACV in vitro [4,6,29]. Future studies comparing various acyclic nucleosides and IFNs against different viruses should yield more definitive answers.

In summary, the present results indicate that DHPG and rMuIFN-γ will potentiate the activity of the other against a systemic infection of HSV-2 in mice. This synergistic interaction may be related to the immunological effects of IFN-γ in addition to the direct interaction of both drugs at the infective site. The possibility of combining DHPG and IFN-γ treatments in the clinic should be considered.

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