ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1984, p. 563–565
0066-4804/84/050563-03$02.00/0

Vol. 25, No. 5

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

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Received 28 November 1983/Accepted 23 February 1984

The acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) and natural mouse interferon beta (MuIFN-β) were evaluated for their efficacy alone and in combination against herpes simplex virus type 2 systemic infections 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 injected subcutaneously at doses of 0.7 to 6 mg/kg. MuIFN-β was given intraperitoneally at doses ranging from $3 \times 10^3$ to $3 \times 10^4$ IU per mouse. For DHPG alone, the effective dose at which 50% of the mice survived ($ED_{50}$) was >6 mg/kg. However, when given in combination with an ineffective dose of MuIFN-β ($10^4$ IU per mouse), the $ED_{50}$ for DHPG was 0.8 mg/kg. In addition, at the highest dose tested, MuIFN-β alone had no protective activity against herpes simplex virus type 2 ($ED_{50}$, $>3 \times 10^4$ IU per mouse). However, when given in combination with a marginally effective dose of DHPG (2 mg/kg), the $ED_{50}$ for MuIFN-β was $<3 \times 10^3$ IU per mouse. Calculation of the fractional protective dose index (<0.23 where values of ≤0.5 are considered synergistic) indicates an enhanced protective interaction by the combination of the two drugs. These results represent the first time that potentiation of the antiviral activity of an acyclic nucleoside by interferon has been demonstrated in animal studies.

The acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) is a promising new antiviral agent. This compound has good protective activity against both systemic and local infections of the skin or genital tract caused by herpes simplex virus (HSV) (1, 5, 7, 20). DHPG is also known as BIOLF-62 (21), 2'NDG (1), and BW759 (18), depending on its source.

The immune regulatory and antiviral agent interferon (IFN) has also provided some efficacy against HSV in recent animal studies. Protective activity against this virus has been reported for both systemic and local infections of the eye, skin, and genital tract (10, 11; J. C. Overall, Jr., T. J. Yeh, and E. R. Kern, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 392, 1983). In addition, in human clinical trials, both local and systemic infections of HSV have also benefited from IFN treatments (13, 17). At present, all three types of IFN, which encompass molecular and biological differences, are of potential interest: α (leukocyte), β (fibroblast), and γ (immune).

Recently, combinations of different IFNs and either DHPG or 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine, known as acyclovir [ACV]) have been found to be additive to synergistic against HSV grown in vitro (9, 12, 22; 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; D. A. Eppstein and Y. V. Marsh, Biochem. Biophys. Res. Commun., in press). Thus, since DHPG or IFN can be effective in treating in vivo HSV infections when used alone, it is possible that they would have an enhanced antiviral action if given together, as was seen in vitro. To test this hypothesis, a systemic HSV infection of mice was treated with DHPG or murine IFN-β (MuIFN-β) alone or in combination, and survival was monitored. In this model, death results from encephalitis after an initial multiplication of virus in several visceral organs (16).

## MATERIALS AND METHODS

**Compounds.** DHPG was synthesized as described previously (14). The dry powder was solubilized fresh daily in physiological saline before administration. The preparation was sonicated for 30 s (probe model no. H-1; Ultrasonics, Inc., Plainview, N.Y.) to speed up solubilization.

Natural MuIFN-β was purchased from Lee Biomolecular, San Diego, Calif. Antiviral activity was determined by a microtiter inhibition of cytopathic effect assay using mouse fibroblast ($L_{929}$) cells, vesicular stomatitis virus, and National Institutes of Health reference reagent MuIFN(α,β) no. G002-094-511 (23). The specific activity of the MuIFN-β preparation was $2 \times 10^8$ IU/mg. Greater than 99.6% of this antiviral activity was neutralized with specific anti-MuIFN-β serum as determined by Lee Biomolecular. The MuIFN-β was solubilized in physiological buffered saline with 0.1 mg of mouse serum albumin (MSA; Sigma Chemical Co., St. Louis, Mo.) per ml.

**Virus and cells.** HSV type 2 (HSV-2), G strain, was propagated in human laryngeal carcinoma (HEp-2) cells and plaque titrated in African green monkey (Vero) cells. The HSV-2 and HEp-2, Vero, and $L_{929}$ cells were obtained from the American Type Culture Collection, Rockville, Md. The vesicular stomatitis virus (Indiana strain) was obtained from C. E. Samuel, University of California at Santa Barbara.

**Animals and infection.** Female Swiss Webster mice (Charles River Breeding Laboratories, Wilmington, Mass.) of 14 to 17 g (weight range) were used for the infection. The animals were inoculated intraperitoneally with $10^3$ PFU of HSV-2 and randomized into 14 groups of 20 mice each. This HSV-2 challenge was approximately four 50% lethal doses

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and consistently produced 100% mortality in saline-treated controls.

**Treatment with antiviral compounds.** Starting 24 h postinfection, 12 groups of 20 mice were treated with different doses of MuIFN-β and/or DHPG. MuIFN-β was injected intraperitoneally, and DHPG was injected subcutaneously. Both compounds were given within an hour of each other once a day for 5 days. The remaining two groups of 20 mice served as controls. These control animals were treated with either saline (DHPG control) or saline with MSA (MuIFN-β control). Mice were observed for mortality for 21 days after challenge.

**Statistical analyses.** Statistical evaluation of differences in the number of animals which survived the infection was done by a two-tailed Fisher exact probability test (15). Probit analysis (6) was used to calculate the effective dose of MuIFN-β or DHPG at which 50% of the mice survived ($ED_{50}$). $ED_{50}$s were obtained for MuIFN-β or DHPG alone, for MuIFN-β with 2 mg of DHPG per kg, and for DHPG with $10^4$ IU of MuIFN-β per mouse. Differences in survival time between the various treated and control groups were analyzed with the Mann-Whitney U probabilty test (8). All mice which survived to the end of the test (day 21) were assigned a survival time of 1 day after the test had ended, i.e., day 22, for this comparison.

**FPDI.** The possibility of a synergistic interaction between drugs was analyzed by calculating the fractional protective dose index (FPDI) (2). The FPDI was calculated as ($ED_{50}$ of drug A in combination/$ED_{50}$ of drug A alone) plus ($ED_{50}$ of drug B in combination/$ED_{50}$ of drug B 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.

TABLE 1. Survival of mice treated with different doses of DHPG or MuIFN-β alone, DHPG in combination with an ineffective dose of MuIFN-β ($10^4$ IU per mouse), or MuIFN-β in combination with a marginally effective concentration of DHPG (2 mg/kg) against intraperitoneal HSV-2 infection

| Treatment regimen | | Response to treatment[a] | | $ED_{50}$ | |
|---|---|---|---|---|---|
| DHPG (mg/kg) | MuIFN-β (IU/mouse) | No. surviving mice/total | Survival time[b] (days) | DHPG (mg/kg) | MuIFN-β (IU/mouse) |
| Saline | | 0/19 | 9.0 | | |
| 0.7 | 0 | 3/20 | 12.9[c] | | |
| 2 | 0 | 4/20 | 13.3[c] | >6 | |
| 6 | 0 | 8/20[c] | 17.2[c] | | |
| 0.7 | $10^4$ | 9/20[c] | 17.6[c] | | |
| 2 | $10^4$ | 17/19[c] | 21.3[c] | 0.8[d] | $10^4$[d] |
| 6 | $10^4$ | 20/20[c] | 22.0[c] | | |
| | Saline/MSA | 0/20 | 10.3 | | |
| 0 | $3 \times 10^3$ | 1/20 | 9.3 | | |
| 0 | $10^4$ | 2/20 | 10.7 | | $>3 \times 10^4$ |
| 0 | $3 \times 10^4$ | 2/20 | 10.7 | | |
| 2 | $3 \times 10^3$ | 11/18[c] | 18.7[c] | | |
| 2 | $10^4$ | 17/20[c] | 20.6[c] | 2[d] | $<3 \times 10^3$[d] |
| 2 | $3 \times 10^4$ | 17/20[c] | 21.2[c] | | |

[a] Over a 21-day observation period. All surviving mice were healthy at the end of this time.

[b] All mice which survived to the end of the test (day 21) were assigned a survival time of 1 day after the test had ended (day 22) for this comparison.

[c] Significantly different ($P < 0.05$) from saline- or saline/MSA-treated controls.

[d] $ED_{50}$s are given for DHPG and MuIFN-β in combination.

## RESULTS

Table 1 shows the effect of MuIFN-β on the antiviral activity of DHPG as measured by survival of mice. At the concentration used ($10^4$ IU per mouse), MuIFN-β alone had no significant protective activity against HSV-2. Only 10% of mice survived, and the survival time was comparable to the saline/MSA-treated control. However, when used in combination with various doses of DHPG, the drug potentiated the activity of DHPG against HSV-2. The $ED_{50}$ for DHPG alone was >6 mg/kg. In combination with the ineffective dose of MuIFN-β, the $ED_{50}$ for DHPG was lowered about 10-fold to 0.8 mg/kg.

Table 1 also shows the effect of DHPG on the antiviral activity of MuIFN-β as measured by survival of mice. By itself, MuIFN-β was ineffective against HSV-2, even at the highest dose tested ($3 \times 10^4$ IU per mouse [only 10% survivors]). DHPG alone at the concentration used (2 mg/kg) also had little protective activity on ultimate survival against HSV-2 (only 20% survivors). However, when used in combination with the various ineffective doses of MuIFN-β, the drug potentiated MuIFN-β activity against HSV-2. The $ED_{50}$ for MuIFN-β alone was $>3 \times 10^4$ IU per mouse. In combination with the marginally effective dose of DHPG, the $ED_{50}$ for MuIFN-β was lowered more than 10-fold to $<3 \times 10^3$ IU per mouse.

A synergistic interaction between these two drugs was confirmed by calculating the FPDI value from the $ED_{50}$s of the drugs alone and in combination. This analysis yielded a FPDI of 0.23, where a value of ≤0.5 indicates potentiation. Since the actual $ED_{50}$s were greater than the highest dose used for DHPG alone (>6 mg/kg) and for MuIFN-β alone ($>3 \times 10^4$ IU per mouse) and less than the lowest dose for MuIFN-β in combination ($<3 \times 10^3$ IU per mouse), the actual FPDI value would be even lower than 0.23.

## DISCUSSION

To our knowledge, these results represent the first time that potentiation of the antiviral activity of an acyclic nucleoside by IFN has been demonstrated in animal studies. Recently, Rose et al. (19) 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. More recently, Wade et al. (24) treated CMV pneumonia in humans with high-dose ACV and human leukocyte IFN. This combination was also 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 (3, 5, 20). Future studies comparing various acyclic nucleosides and IFNs against different viruses should yield more definitive answers.

In a recent clinical trial, Colin et al. (4) tested ACV in combination with human leukocyte IFN against herpes keratitis. An increased antiviral activity was found with the combination, but there were not enough data to determine whether the combination was only additive or actually synergistic. For example, no data were presented on the effect of the IFN-α alone on the course of the disease.

In summary, the present results indicate that DHPG and MuIFN-β will potentiate the activity of the other against a systemic infection of HSV-2 in mice. Hopefully, such a combination with human IFN-β will prove useful against serious HSV infections in clinical situations.

## ACKNOWLEDGMENTS

We thank Ed Halol and Stan Bingham for technical assistance and J. P. H. Verheyden and J. C. Martin for providing the DHPG.

## LITERATURE CITED

1. **Ashton, W. T., J. D. Karkas, A. K. Field, and R. L. Tolman.** 1982. Activation by thymidine kinase and potent antiherpetic activity of 2′-nor-2′-deoxyguanosine (2′NDG). Biochem. Biophys. Res. Commun. **108**:1716–1721.
2. **Berenbaum, M. C.** 1977. Synergy, additivism and antagonism in immunosuppression: a critical review. Clin. Exp. Immunol. **28**:1–18.
3. **Cheng, Y.-C., E.-S. Huang, J.-C. Lin, E.-C. Mar, J. S. Pagano, G. E. Dutschman, and S. P. Grill.** 1983. Unique spectrum of activity of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine against herpesviruses in vitro and its mode of action against herpes simplex virus type 1. Proc. Natl. Acad. Sci. U.S.A. **80**:2767–2770.
4. **Colin, J., C. Chastel, G. Renard, and K. Cantell.** 1982. Herpes oculaire activite antivirale synergique de l'acyclovir et de l'interferon leucocytaire humain. Nouv. Presse Med. **11**:2783.
5. **Field, A. K., 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.** 1983. 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine: a selective inhibitor of herpes group virus replication. Proc. Natl. Acad. Sci. U.S.A. **80**:4139–4143.
6. **Finney, D. J.** 1964. Probit analysis, 2nd ed. Cambridge University Press, England.
7. **Fraser-Smith, E. B., D. F. Smee, and T. R. Matthews.** 1983. Efficacy of the acyclic nucleoside, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, against primary and recrudescent genital herpes infections in guinea pigs. Antimicrobial. Agents Chemother. **24**:883–887.
8. **Hollander, N., and D. A. Wolfe.** 1973. Nonparametric statistical methods. John Wiley & Sons, Inc., New York.
9. **Janz, C., and R. Wigand.** 1982. Combined interaction of antiherpes substances and interferon beta on the multiplication of herpes simplex virus. Arch. Virol. **73**:135–143.
10. **Kern, E. R., and L. A. Glasgow.** 1981. Evaluation of interferon and interferon inducers as antiviral agents: animal studies. Pharmacol. Ther. **13**:1–38.
11. **Kohl, S.** 1983. Stimulation of human natural killer cytotoxicity and protection of mice from infection due to herpes simplex virus by recombinant human leukocyte interferon. J. Infect. Dis. **147**:484–488.
12. **Levin, M. J., and P. L. Leary.** 1981. Inhibition of human herpesviruses by combination of acyclovir and human leukocyte interferon. Infect. Immun. **32**:995–999.
13. **Levin, S.** 1983. Interferon in acute viral infections. Eur. J. Pediatr. **140**:2–4.
14. **Martin, J. C., C. A. Dvorak, D. F. Smee, T. R. Matthews, and J. P. H. Verheyden.** 1983. 9-[(1,3-dihydroxy-2-propoxy)methyl]-guanine: a new potent and selective antiherpes agent. J. Med. Chem. **26**:759–761.
15. **Maxwell, A. E.** 1961. Analyzing quantitative data, p. 13–23. Spottiswoode, Ballantyne and Co., Ltd., London.
16. **Olsen, G. A., E. R. Kern, J. C. Overall, Jr., and L. A. Glasgow.** 1978. Effect of treatment with exogenous interferon, polyriboinosinic-polyribocytidylic acid or polyriboinosinic-polyribocytidylic acid-poly-L-lysine complex on herpesvirus hominis infection in mice. J. Infect. Dis. **137**:428–436.
17. **Pazin, G. J., J. A. Armstrong, M. T. Lam, G. C. Tarr, P. J. Janetta, and M. Ho.** 1979. Prevention of reactivated herpes simplex infection by human leukocyte interferon after operation on the trigeminal root. N. Engl. J. Med. **301**:225–230.
18. **Rollinson, E. A., and G. White.** 1983. Relative activities of acyclovir and BW759 against Aujeszky's disease and equine rhinopneumonitis viruses. Antimicrob. Agents Chemother. **24**:221–226.
19. **Rose, R. M., C. Crumpacker, J. L. Waner, and J. D. Brain.** 1983. Treatment of murine cytomegalovirus pneumonia with acyclovir and interferon. Am. Rev. Respir. Dis. **127**:198–203.
20. **Smee, D. F., J. C. Martin, J. P. H. Verheyden, and T. R. Matthews.** 1983. Anti-herpesvirus activity of the acyclic nucleoside, 9-(1,3-dihydroxy-2-propoxymethyl)guanine. Antimicrob. Agents Chemother. **23**:676–682.
21. **Smith, K. O., K. S. Galloway, W. L. Kennell, K. K. Ogilvie, and B. K. Radatus.** 1982. A new nucleoside analog, 9{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine, highly active in vitro against herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. **22**:55–61.
22. **Stanwick, T. R., R. F. Schinazi, D. E. Campbell, and A. J. Nahmias.** 1981. Combined antiviral effect of interferon and acyclovir on herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. **19**:672–674.
23. **Stewart, W. E. II.** 1979. The interferon system, p. 13–26. Springer-Verlag, New York.
24. **Wade, J. C., R. W. McGuffin, S. C. Springmeyer, B. Newton, J. W. Singer, and J. D. Meyers.** 1983. Treatment of cytomegaloviral pneumonia with high-dose acyclovir and human leukocyte interferon. J. Infect. Dis. **148**:557–562.