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Synergistic Effect of Human Leukocyte Interferon and Nonoxynol 9 against Herpes Simplex Virus Type 2

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The nonionic surfactant nonoxynol 9 (NP9), in combination with human alpha interferon, synergistically reduced the titer of herpes simplex virus type 2 (HSV-2) in vitro. The degree of synergy was highest at an interferon concentration of 10^3 IU/ml and an NP9 dilution of 1:1,500. We postulate that NP9 inactivates extracellular HSV-2, whereas interferon inhibits HSV-2 replication at the intracellular level.

Very high doses of interferon (IFN) are required for treatment of diseases caused by herpesviruses. To increase the clinical efficacy of IFN therapy, we supplemented IFN with another antiviral agent possibly having a different mechanism of action. It has been reported that IFN inhibits herpesviruses synergistically when combined with acyclovir or other antiviral agents (2, 5, 7, 10). We selected nonoxynol 9 (NP9), a nonionic surfactant detergent that acts at the interface between the lipid-containing membrane and the surrounding aqueous medium. NP9 is known to have antiherpetic activity in vitro (1), which appears to result from the disruption of the virus envelope and the nucleocapsid due to ether or amide linkages between the hydrophilic and hydrophobic portions of the molecule. Nonionic surfactants have proven spermicidal activity and are the active ingredients in over-the-counter contraceptive creams and foams. Despite the obvious toxic effect of surfactants on cells in vitro (1), topical treatment has not demonstrated major adverse effects in humans. The results of preliminary trials with NP9 against herpetic infection in patients have been ambiguous (3, 11). As an initial step for later in vivo studies, we wanted to examine the combined antiviral effect of NP9 and human IFN (Hu IFN) against herpes simplex virus type 2 (HSV-2) in vitro.

HSV-2 strain 333 (a human isolate) was originally obtained from W. E. Rawls, McMaster University, Hamilton, Ontario, Canada. Virus was plaque purified and grown in human embryo lung (HEL) cells. HEL cells were propagated in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (MBA Bioproducts), glutamine, and kanamycin. Virus titrations were performed in HEL cells by plaque assay, using a 0.5% methylcellulose overlay at 37°C in an atmosphere of 5% CO_2 (8). Vesicular stomatitis virus Indiana strain was propagated in HEL cells and was used as a challenge virus for titration of IFN.

Hu IFN-α lot no. 40881-10 (Life Sciences, Inc., St. Petersburg, Fla.) was used in the experiments described. The antiviral titer of the IFN was determined by plaque inhibition assays with vesicular stomatitis virus as the challenge virus. Briefly, confluent HEL cells in 35-mm plates were pretreated for 24 h with serial twofold dilutions of IFN. Cells were infected with approximately 40 PFU of vesicular stomatitis virus per culture plate and were fixed 18 h later. IFN titers were determined according to the formula of Langford et al. (6) and were expressed as 1-ml volumes corrected against National Institutes of Health Hu IFN-α reference reagent no. G023-901-527 (distributed by the Reference Reagents Board of the National Institute of Allergy and Infectious Diseases, Bethesda, Md.). NP9 was obtained from Union Carbide Corp., New York, N.Y.

Confluent HEL cells propagated in 35-mm plates were pretreated for 24 h with appropriate concentrations of IFN (1 ml per plate). Different dilutions of NP9 prepared in Tris buffer were mixed with the HSV-2 suspension and rocked at room temperature for 30 s. Serial 10-fold dilutions of this mixture were prepared with Tris buffer and plated on HEL cells either pretreated or not treated with IFN (0.2 ml per plate, two plates per virus dilution). The plates were rocked at room temperature for 1 h, and the cultures were overlaid with 0.5% methylcellulose. After incubation for 46 h at 37°C, the cells were fixed, and the plaques were then counted. The degree of synergy was calculated by the formula of Spector et al. (9), dividing the expected value by the experimental value. The expected value was determined with the formula $A(1 + 2) = (A1 \times A2)/VC$, where $A1$ is the virus titer after

TABLE 1. Effect of increasing dilutions of NP9 and Hu IFN-α against HSV-2[a]

Dilution of NP9	Virus titer[b] (PFU/ml) after treatment with:			Expected titer[c] (PFU/ml)	Degree of synergy[c]
	NP9	Hu IFN-α	NP9 + Hu IFN-α		
		3.0×10^6			
1:500	$>5.0 \times 10^1$	3.0×10^6	$>5.0 \times 10^1$		
1:1,000	2.0×10^4	3.0×10^6	2.5×10^2	5.0×10^3	6.0
1:1,500	5.0×10^6	3.0×10^6	2.0×10^4	3.8×10^5	18.75
1:2,000	2.0×10^7	3.0×10^6	1.0×10^6	1.5×10^6	1.5
1:2,500	4.0×10^7	3.0×10^6	5.0×10^6	3.0×10^6	0.6

[a] HEL cells were pretreated with 10^3 IU of Hu IFN-α per ml, washed, and infected with HSV-2 treated with NP9. Mixtures of different dilutions of NP9 (1:500, 1:1,000, 1:1,500, 1:2,000, and 1:2,500) and undiluted virus stock suspension were kept at room temperature for 30 s. Serial 10-fold dilutions were prepared, adsorbed to HEL cells (two 35-mm plates per dilution) for 1 h at room temperature, and overlaid with semisolid medium. Cells were fixed after 46 h at 37°C.

[b] Virus control titer was 4.0×10^7 PFU/ml.

[c] Expected titer and degree of synergy were calculated according to the formula described by Spector et al. (9).

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TABLE 2. Effect of increasing doses of Hu IFN-α and NP9 against HSV-2[a]

Hu IFN-α (IU/ml)	Virus titer[b] (PFU/ml) after treatment with:			Expected titer[c] (PFU/ml)	Degree of synergy[c]
	NP9	Hu IFN-α	NP9 + Hu IFN-α		
	3.5×10^4				
10^1	3.5×10^4	1.1×10^8	1.0×10^4	2.75×10^4	2.75
10^2	3.5×10^4	7.0×10^7	1.5×10^3	1.75×10^4	11.67
10^3	3.5×10^4	3.0×10^7	4.0×10^2	7.50×10^3	18.75
10^4	3.5×10^4	5.0×10^6	6.5×10^2	1.25×10^3	1.92

[a] HEL cells were pretreated with 10^1, 10^2, 10^3, and 10^4 IU of Hu IFN-α per ml for 24 h, washed, and infected with HSV-2 treated with NP9. The mixture of NP9 (1:1,500 dilution) and undiluted virus stock were kept at room temperature for 30 s. Serial 10-fold dilutions were prepared, and 0.2 ml per plate (two plates per dilution) was adsorbed to HEL cells for 1 h at room temperature and overlaid with semisolid medium. Cells were fixed after 46 h at 37°C.

[b] Virus control titer was 2.0×10^8 PFU/ml.

[c] Expected titer and degree of synergy were calculated according to the formula described by Spector et al. (9).

inactivation with agent 1; *A*2 is the virus titer after inactivation with agent 2; *A*(1 + 2) is the virus titer after inactivation with both agents; and *VC* is the control virus titer.

To determine whether different dilutions of NP9 combined with IFN acted synergistically, dilutions of 1:500, 1:1,000, 1:1,500, 1:2,000, and 1:2,500 of NP9 were tested in combination with 10^3 IU of Hu IFN-α per ml against HSV-2 (Table 1). We observed synergistic effects at all combinations tested, with the greatest degree of synergy at a dilution of 1:1,500. HSV-2 was then tested with 10^1, 10^2, 10^3, or 10^4 IU of Hu IFN-α per ml combined with a dilution of 1:1,500 of NP9. Synergism was observed at all combinations tested; again, however, the greatest degree of synergy was observed with 10^3 IU of Hu IFN-α per ml (Table 2).

Different dilutions of NP9 (1:750, 1:1,000, 1:1,500, 1:2,000, and 1:2,500) combined with recombinant IFN-αA (10^3 IU/ml; supplied by Hoffmann-La Roche Inc., Nutley, N.J.) were also tested for synergism against HSV-2. The greatest degree of synergy with these reagents was found at a dilution of 1:1,000 of NP9 combined with 10^3 IU of IFN-αA per ml (Table 3).

TABLE 3. Synergistic effect of IFN-αA and NP9 on HSV-2[a]

Dilution of NP9	Virus titer[b] (PFU/ml) after treatment with:			Expected titer[c] (PFU/ml)	Degree of synergy[c]
	NP9	IFN-αA	NP9 + IFN-αA		
		2.5×10^6			
1:750	1.5×10^4	2.5×10^6	1.5×10^3	8.3×10^2	0.56
1:1,000	1.0×10^5	2.5×10^6	1.0×10^3	5.5×10^3	5.56
1:1,500	5.0×10^5	2.5×10^6	1.0×10^5	2.7×10^4	0.28
1:2,000	1.0×10^7	2.5×10^6	5.0×10^5	5.5×10^5	1.11
1:2,500	3.5×10^7	2.5×10^6	2.0×10^6	1.9×10^6	0.97

[a] HEL cells were pretreated with 10^3 IU of recombinant IFN-αA per ml, washed, and infected with HSV-2 treated with NP9. Mixtures of different dilutions of NP9 (1:750, 1:1,000, 1:1,500, 1:2,000, and 1:2,500) and undiluted virus stock were kept at room temperature for 30 s. Serial 10-fold dilutions were prepared, adsorbed to HEL cells (two 35-mm plates per dilution) for 1 h at room temperature, and overlaid with semisolid medium. Cells were fixed after 46 h at 37°C.

[b] Virus control titer was 4.5×10^7 PFU/ml.

[c] Expected titer and degree of synergy were calculated according to the formula described by Spector et al. (9).

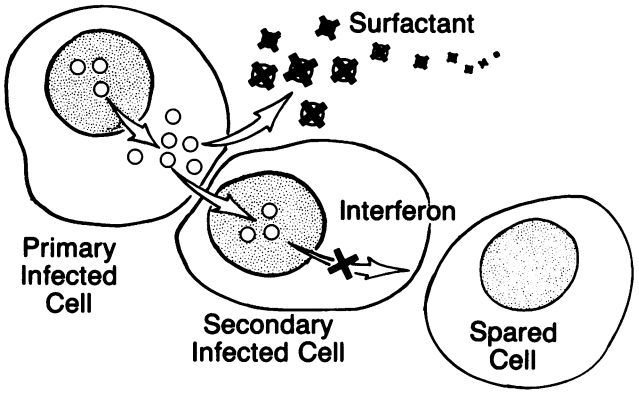

FIG. 1. Postulated mechanism of synergistic action of surfactant and IFN against HSV-2.

The results of these experiments clearly show that Hu IFN-α combined with NP9 inhibited HSV-2 synergistically in vitro. Interestingly, the synergy index was highest only at a narrow range of concentrations for either compound: 10^2 to 10^3 IU of Hu IFN-α per ml and at dilutions of 1:1,000 to 1:1,500 for NP9. The mechanism of the synergistic action on HSV-2 by both compounds is unknown. It is known that surfactants inactivate HSV-2 by solubilizing the virus envelope, and the mechanism of inhibition of HSV-2 by IFN is presumably through an intracellular antiviral protein. Our working hypothesis is that the surfactant destroys extracellular virus and IFN inhibits replication of virus at the intracellular level (Fig. 1). This differs from the mechanism of synergistic inactivation of HSV-2 by 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine combined with recombinant interferons that has been offered by Eppstein and Marsh (4), who proposed that 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine and IFN act intracellularly, although at different stages of virus replication.

The degree of synergy we observed for inactivation of HSV-2 by Hu IFN-α and NP9 in vitro is relatively high. The synergistic effect of Hu IFN-α and NP9 warrants in vivo trials as a topical treatment, with careful attention to the concentrations of both compounds.

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