VIROLOGY

Combined Antiherpetic Effect of Complex Preparation "Viferon® — Eye Drops" and Modified Nucleosides

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 672-675, June, 2006
Original article submitted June 20, 2005

Ready dosage form (eye drops) prepared on the basis of recombinant α_2-IFN exhibits high activity towards herpes simplex type 1 virus *in vitro*. Systematic study of the antiherpesvirus effect of this drug in combination with modified nucleosides showed an inhibitory effect of the synergic type. Combination of IFN preparation with some nucleosides, including ribavirin, proved to be highly effective towards drug-resistant herpes virus.

Key Words: *herpes simplex virus; α_2-interferon ready dosage form; modified nucleosides; combined chemotherapy, cell culture*

The role of recombinant IFN as a drug for the therapy of prevalent and socially significant infections is increasing. Dosage forms created on the basis of recombinant IFN are used for systemic treatment in medical practice, for example, as injections and suppositories [2,4].

Creation of new IFN-based dosage forms for ophthalmology and otorhinolaryngology is an important task.

Interferon in combination with modified nucleosides (viral DNA and RNA synthesis inhibitors) is used as an antiviral drug [2]. This use of IFN is the most perspective for the development of herpesvirus infection chemotherapy. We studied antiviral efficiency of a combination of recombinant α_2-IFN ready dosage form (IFN RDF) with modified nucleosides used in modern medicine.

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MATERIALS AND METHODS

"Viferon® — eye drops" IFN RDF (composition: recombinant α_2-IFN (40×10^3 U/ml), Vector-Farm Company), acyclovir (zovirax; Wellcome), gancyclovir (cimevene; Roche), 9-β-D-arabinofuranosyladenine (Ara A; vidarabine), 5-iodo-2'-deoxyuridine (IDU), (E)-5-(bromovinyl)-2'-deoxyuridine (BVDU; Sigma), ribavirin (virasole; ICN Pharmaceutical), phosphoformic acid (PFA) as trisodium salt (foscarnet; Astra) were used in the study.

Experiments were carried out on Vero E6 cells, Eagle's medium with 7% FCS served as growth medium. The following virus strains were used: type 1 herpes simplex virus (HSV-1) strain L_2 highly sensitive to acyclovir and gancyclovir; HSV-1 clinical strain Lab highly sensitive to acyclovir (IE_{50} 0.45 μg/ml), gancyclovir, and Ara A; HSV-1 clinical strain Shash with sharply reduced sensitivity to acyclovir (IE_{50} 25 μg/ml) and sensitive to Ara A and PFA.

The routine method for evaluation of antiviral activity of compounds (CPE inhibition assay [1,6,

7]) is based on determination of the inhibitory concentration of the compound preventing the development of virus-induced cytopathogenic effect by 50% (IE_{50}) and by 90-100% (IE_{95}). The cells were cultured in 96-well plastic plates and infected in a dose of 0.1 TCE_{50}/cell. The cells were incubated for 48-72 h at 37°C and 5% CO_2. Under these conditions the cytopathic effect involving the entire cell monolayer developed in the control sample. The preparations were added directly after cell culture infection. Antiviral effect of IFN RDF with modified nucleosides was evaluated by the common method, including estimation of the fraction inhibitory concentration index [1,5].

The cytotoxicity of compounds was studied during 96 h by the method based on estimation of the counts of live and trypan blue-stained [1,6] dead cells and calculation of the CE_{50} (concentration at which at least 50% cells survived in comparison with the control).

RESULTS

Anti-HSV effects of α_2-IFN and its drop form RDF are presented in Table 1. The preparation was highly effective *in vitro*, its activity being similar to that of the parental IFN sample even towards the virus strain with significantly reduced sensitivity to acyclovir and some other modified nucleosides. IFN-containing preparations also exhibited high activity towards HSV Lab clinical strain. Placebo preparation containing no IFN is characterized by minimum viral activity, more than 15-fold lower than that of ready dosage form.

All test IFN preparations are characterized by selective anti-HSV effect in concentrations not toxic for Vero E6 cell culture (Table 1).

Previous *in vitro* studies revealed a synergic anti-HSV effect of α_2-IFN in combination with acyclovir and pencyclovir [5]. We found that combination of IFN drop RDF with all test preparations

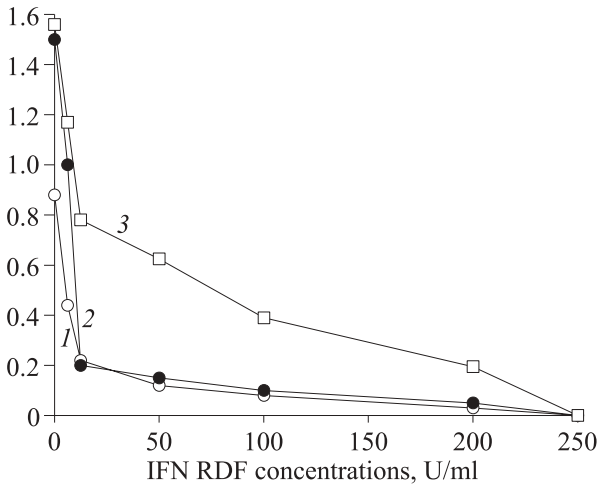

Fig. 1. Dynamics of combined anti-HSV effect of Viferon^R eye drops (IFN RDF) with gancyclovir, 5-iodo-2'-deoxyuridine (IDU), or phosphoformic acid (PFA) in Vero E6 cell culture (HSV-1 strain L_2). Ordinate: concentrations of gancyclovir and IDU (μg/ml), PFA (μg/0.1 ml). *1*) IFN RDF+gancyclovir; *2*) IFN RDF+IDU; *3*) IFN RDF+PFA.

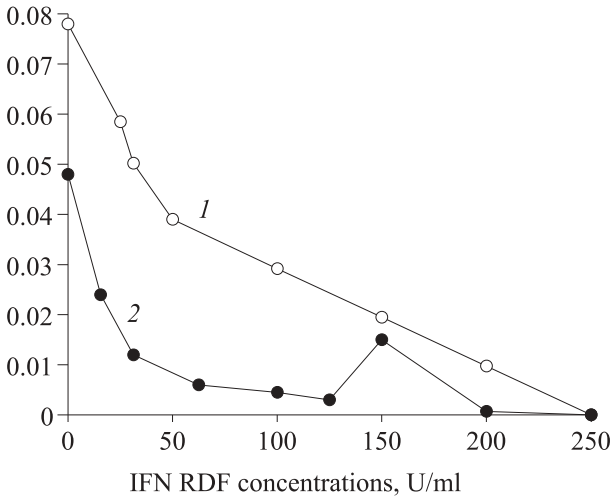

Fig. 2. Combined anti-HSV effect of IFN RDF and Ara A or BDVU in Vero E6 cell culture (HSV-1 strain L_2). Ordinate: concentrations of BVDU (μg/ml) and Ara A (μg/0.1 ml). *1*) IFN RDF+Ara A; *2*) IFN RDF+BVDU.

TABLE 1. Anti-HSV Activity of IFN Drop RDF in Vero E6 Cell Culture

Preparation	CE_{50}	HSV-1 strain			
		L_2		Shash	
		IE_{50}	IE_{95}	IE_{50}	IE_{95}
IFN, U/ml	$>10\times10^6$	9.62×10^2	6.20×10^4	9.62×10^2	6.20×10^4
IFN RDF, Unit/ml	$>8\times10^3$	2.50×10^2	4.00×10^3	2.50×10^2	4.00×10^3
Acyclovir, μg/ml	>400	0.4	0.95	25.0	50.0
Ara A, μg/ml	80.0	7.8	15.6	7.8	15.6

TABLE 2. Estimated Indexes of Fraction Inhibitory Concentrations (FIC) for IFN RDF Combinations with Modified Nucleosides

Combination	FIC	Inhibition type
IFN RDF+acyclovir	0.23	FIC<0.5: pronounced synergism
IFN RDF+gancyclovir	0.34	FIC<0.5: pronounced synergism
IFN RDF+IDU	0.30	FIC<0.5: pronounced synergism
IFN RDF+BVDU	0.37	FIC<0.5: pronounced synergism
IFN RDF+Ara A	0.70	0.5<FIC<0.9: weak synergism
IFN RDF+ribavirin	0.50	<0.5: synergism
IFN RDF+PFA	0.55	0.5<FIC<0.9: weak synergism

Note. FIC index was calculated by the formula: $\frac{IE_{50} \text{ for compound A in combination}}{IE_{50} \text{ for compound B in combination}} + \frac{IE_{50} \text{ for compound A}}{IE_{50} \text{ for compound B}}$.

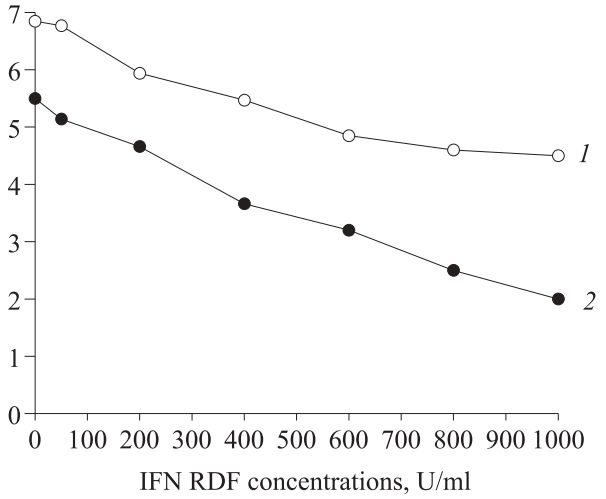

Fig. 3. Effects of IFN RDF and its combination with ribavirin on infective titer of acyclovir-resistant HSV-1 strain Shash (IE_{50} 25 μg/ml) in Vero E6 cell culture. Ordinate: HSV infective titer (lg TCE_{50}/ml). *1*) IFN RDF; *2*) IFN RDF+ribavirin.

provided a synergic anti-HSV effect (for HSV L_2 model). Due to combination of acyclovir with IFN RDF, IFN concentration was reduced 40-fold and that of acyclovir 133 times. Gancyclovir IE_{50} decreased 29 times in combined use, that of IFN RDF 40 times (Fig. 1). Similar results were obtained for combination of an IFN-containing preparation with IDU (Fig. 1), BVDU (Fig. 2), and ribavirin. The synergic antiviral effect of combinations with Ara A (Fig. 2) and PFA (Fig. 1) was the minimum, the antiviral effect approaching the additive.

Indexes of fraction inhibitory concentrations confirmed our results (Table 2). The highest effect was exhibited by IFN RDF combination with acyclovir, the least by its combination with Ara A.

Ribavirin, Ara A, and PFA showed antiviral activity towards HSV variants resistant to acyclovir, gancyclovir, IDU, and BVDU. For this reason combined antiviral effect of IFN RDF with ribavirin, Ara A, and PFA was also observed for acyclovir-resistant HSV variants. These were mainly viruses with the thymidine kinase gene-deficient genome [3]. IFN RDF in the presence of the minimum active concentration of ribavirin (150 μg/ml) exhibited a significantly higher anti-HSV effect (Fig. 3). All the studied combinations of compounds exhibited anti-HSV activities being used in non-cytotoxic concentrations.

Our results extend potentialities of using combined therapy in the treatment of HSV infection, including drug-resistant. In this latter case α_2-IFN preparations can be combined with ribavirin, Ara A, or PFA. Combination of recombinant α_2-IFN with ribavirin can be practically significant for the treatment of not only hepatitis C, but also for the therapy of other viral infections, for example, severe acute respiratory syndrome and cattle diarrhea [8,9].

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