# COMBINED ACTION OF SOME NUCLEOSIDES AND α-INTERFERON DURING INHIBITION OF REPRODUCTION OF TYPE II HERPES SIMPLEX VIRUS

E. V. Temicheva, V. V. Malinovskaya, L. S. Manakhova, L. L. Mironova, and G. A. Galegov

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Discovery of mutual enhancement of the antiviral effect of isatin† thiosemicarbazone and phenoxypyrimidines in mice with vaccine encephalitis [9]‡ laid the foundations of the study of drug combinations in several different virus infections. Many investigators showed [2, 4, 7] that a combination of drugs is much more effective if the substances composing it differ in their mechanism of action. The advantage of combinations of chemotherapeutic agents is that inhibition of virus replication is achieved with the use of subtoxic or even nontoxic doses of drugs. The development of resistant mutants of viruses becomes unlikely when combinations of drugs are used. Besides the combined use of inhibitors of viral DNA synthesis, combinations of nucleosides with α- and β-interferons have recently begun to be studied [4, 5, 7]. Investigations of various combinations of antiherpetic agents is a promising trend in the development of methods of treatment of herpetic infection.

This paper describes the study of combinations of acyclovir (ACV), 9-β-D-arabinofuranosyl-adenine (Ara-A), E-5'-(2-bromovinyl)-2'-deoxyuridine (BVDU), and α-interferon on reproduction of type II herpes simplex virus (SHV-II).

## EXPERIMENTAL METHOD

ACV was obtained from M. Yu. Lidak (Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR) [1], Ara-A from A. I. Zinchenko (Institute of Microbiology, Academy of Sciences of the Belorussian SSR, Minsk) [3], and BVDU from I. A. Mikhailopulo (Institute of Bioorganic Chemistry, Academy of Sciences of the Belorussian SSR, Minsk). The substances were used in solution; α-interferon, a lyophilized preparation with specific activity of $10^4$ IU/ml, was dissolved before use (it was obtained from the Department of Interferon Biosynthesis, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR).

HSV-II of the MS strain, adapted to a culture of human diploid cells, was used. The titer of the virus was $10^{4.5}$ $TCD_{50}$/0.1 ml. A diploid line of human fibroblasts of strain M-19 was cultured in Eagle's medium with a double set of amino acids and vitamins and with 2% embryonic calf serum. The antiviral action of the preparations was tested in M-19 microcultures. The cells were introduced into 96-well micropanels in a dose of 30,000 cells/0.1 ml. After monolayer formation the cells were infected with HSV-II, with a multiplicity of infection of 100 $TCD_{50}$, and incubated for 60 min at 37°C. Different concentrations of the test compounds, dissolved in Eagle's medium, were then added. Observation continued for 72 h. The antiviral activity of the inhibitory agents was assessed on the basis of inhibition of the cytopathic action of the virus by 50% — the minimal inhibitory concentration (MIC) compared with the control (100% degeneration of the cells). To obtain an additional estimate of the effectiveness of the combinations of drugs tested a mathematical method [5, 8] was used. This

*Deceased.
†1,3-Indoledione.
‡Not given in Literature Cited — Publisher.

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TABLE 1. Effect of Chemotherapeutic Agents and α-Interferon Separately and in Combinations on Reproduction of HSV-II (Strain MS) in M-19 Cell Culture

| Preparations used | MIC, mg/ml | | | IU/ml | IECP |
|---|---|---|---|---|---|
| | ACV | Ara-A | BVDU | α-Interferon | |
| Separately | 0,1 | — | — | — | — |
| | — | 0,75 | — | — | — |
| In combination (No. of combination) | — | — | 0,16 | — | — |
| | — | — | — | 31 | — |
| 1 | 0,12 | 0,12 | — | — | 1,6 |
| 2 | 0,06 | — | 0,0004 | — | 0,6 |
| 3 | — | 0,7 | 0,006 | — | 0,94 |
| 4 | 0,06 | 0,06 | 0,0007 | — | 0,7 |
| 1 | 0,007 | — | — | 0,07 | 0,072 |
| 2 | — | 1,5 | — | 15 | 2,5 |
| 3 | 0,032 | 0,032 | — | 0,32 | 0,37 |
| 4 | — | 0,065 | 0,0009 | 0,65 | 0,1 |
| 5 | 0,024 | 0,024 | 0,00034 | 0,24 | 0,3 |
| 6 | 0,032 | — | 0,00045 | 0,32 | 0,32 |

Legend. Values of IECP correspond to the following: 0.5) marked synergism; 0.5-1) moderate synergism; 1) additive effect; 1.1-1.9) effects of partial antagonism, 2.0) antagonism. Results of three independent experiments are shown.

consisted of determining the inhibitory index of effectiveness of drug combinations (IEDC) by the following equation:

$$IEDC = \frac{\text{MIC of substance A in combination}}{\text{MIC of substance A separately}} + \frac{\text{MIC of substance B in combination}}{\text{MIC of substance B separately}}.$$

If the value of IEDC was under or equal to 0.5, it corresponded to considerable mutual enhancement of antiviral action of the drugs, if it was 0.5-1 this corresponded to enhancement of antiviral action, and if it was equal to 1, this corresponded to additive interaction of the drugs; if IEDC was 2 or more, this indicated marked antagonism between the actions of the drugs.

Cytotoxicity was determined visually, by the presence or absence of toxic damage to the cells when the antiherpetic agents were used.

## EXPERIMENTAL RESULTS

The therapeutic effect of four combinations of chemotherapeutic agents with antiherpetic action was studied, namely: ACV + BVDU, Ara-A + BVDU, ACU + Ara-A, and ACV + Ara-A + BVDU. With all combinations tested, moderate mutual enhancement of the antiviral effect was observed (Table 1) compared with the activity of each compound separately, except for the combination ACV + Ara-A, with which an effect of partial antagonism was observed. Analysis of MIC showed that the combination ACV + BVDU is optimal, MIC of BVDU was 400 times less in the combination than MIC of the same compound given separately.

Some workers [5] describe the weak inhibitory effect of interferon on HSV-II. The present investigation showed that considerable enhancement of the antiviral action of nucleosides was observed when α-interferon was used as one component of the combination. Altogether six combinations of chemotherapeutic agents with α-interferon were tested (Table 1). The therapeutic effect of combinations of test substances, administered 1 h after infection of the cell culture with the virus, was studied in the present case, whereas many other investigators examined the prophylactic effect of similar combinations [7].

Human inferferon, combined with ACV, Ara-A, and BVDU, exhibited a moderate degree of synergism (in combinations Nos. 3-6, see Table 1). The combination of α-interferon with Ara-A was an example of marked antagonism, as shown by the twofold increase in MIC of Ara-A in the combination compared with MIC for this chemotherapeutic agent when used separately. In a

combination of α-interferon with ACV marked synergism was observed, for MIC of α-interferon in conjunction with ACV was reduced compared with the value of this parameter for each substance separately, by 443 and 14 times respectively. The value of IECP, namely 0.072, also was evidence of the considerable mutual potentiation of the inhibitory action of ACV and α-interferon on HSV-II reproduction. Thus the 10 combinations of Soviet antiherpetic chemotherapeutic agents and α-interferon investigated showed the value of combined treatment of herpetic infection. With respect to antiherpetic activity, all the combinations tested in this investigation can be arranged in the following order: AC + α-interferon > Ara-A + BVDU + α-interferon > ACV + Ara-A + BVDU + α-interferon > ACV + BVDU + α-interferon > ACV + Ara-A + α-interferon > ACV + BVDU > ACV + Ara-A + BVDU > Ara-A + BVDU > ACV + Ara-A > Ara-A + α-interferon.

## LITERATURE CITED


1. O. T. Andreeva, V. I. Votyakov, N. A. Kuzovkina, et al., Chemotherapy and Chemoprophylaxis of Virus Infections: Particularly Dangerous and Slow Infections [in Russian], Minsk (1985), pp. 20-26.
2. G. A. Galegov, V. Knight, N. L. Pushkarskaya, and N. D. L'vov, Vopr. Virusol., No. 6, 69 (1981).
3. G. A. Galegov, S. N. Nikolaeva, R. A. Gibadullin, and V. M. Zhdanov, Genetika, 22, No. 5, 892 (1986).
4. L. V. Allen, L. K. Vanderslice, C. M. Fingal, et al., Antiviral Res., 2, 203 (1982).
5. L. R. Crane, D. A. Milne, J. C. Sunstrum, and A. M. Lerner, Antimicrob. Agents Chemother., 26, 557 (1984).
6. E. De Clercq, J. Descamps, G. Verhelst, et al., J. Infect. Dis., 141, 563 (1980).
7. C. Janz and R. Wigand, Arch. Virol., 73, 135 (1982).
8. M. J. Levin and P. L. Leary, Infect. Immun., 32, 995 (1981).


# PEPTIDOGLYCAN ISOLATED FROM *Lactobacillus bulgaricus*: COMPLEMENT-MEDIATED EFFECT ON MATURATION OF PRECURSOR T CELLS


A. A. Prokop'ev, N. M. Kalinina, S. V. Andreev, A. M. Ishchenko, and S. A. Ketlinskii



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Peptidoglycans (PG) in the cell walls of microorganisms possess immunomodulating activity [1, 11] and can activate the complement system [6]. Fragments of the components of complement formed as a result of activation of the complement system in turn perform the functions of immunoregulators [2, 8]. The immunomodulating action of structural components of the cell walls of microorganisms is realized at the level not only of differentiated lymphocytes, but also of precursors of T and B cells. For example, lipopolysaccharides stimulate T-cell differentiation [10]. The effect of PG isolated from the walls of Gram-positive bacteria on the early stages of T-cell differentiation remains virtually unstudied.

The aim of this investigation was to study the ability of PG from *Lactobacillus bulgaricus* to induce expression of theta-antigen (TAG) on pre-T-lymphocytes of mouse bone marrow and the role of complement as a possible mediator of this induction.

## EXPERIMENTAL METHOD

A bone-marrow suspension from CBA mice was enriched with pre-T-cells on a column with nylon fiber. After concentration the pre-T-lymphocytes ($5 \times 10^6$/ml) were incubated with PG


All-Union Research Institute of Specially Pure Biopreparations, Leningrad.


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