Synergic Antiherpetic Effect of Para-Aminobenzoic Acid and Modified Nucleosides

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New results of combined therapy of herpetic infection in Vero cells infected with herpes simplex type I virus resistant to acyclovir (acycloguanosine, Zovirax) are presented. Para-aminobenzoic acid stimulates antiherpetic effects of acycloguanosine, 5-(2-bromovinyl)-2'-deoxyuridine and adenine 9-β-D-arabinofuranoside. Para-aminobenzoic acid alone is incapable of suppressing type I herpes simplex virus reproduction in cell culture. Modified nucleosides combined with it exert a higher selective antiherpetic effect. Para-aminobenzoic acid is capable of inactivating herpes viruses in culture medium.

Key Words: *herpes simplex virus; modified nucleosides; para-aminobenzoic acid*

Combined chemotherapy of herpetic infection is based on the use of two antiherpetic agents active during different stages of herpesvirus reproduction cycle [4,8]. Modified nucleosides inhibiting production of viral DNA are effective antiherpetic agents [6,9]. Para-aminobenzoic acid (PABA) is a specific antiherpetic compound [1]. Even ineffective concentrations of acycloguanosine (ACG) in combination with PABA completely suppress virus-induced cytopathic effect in Vero cell culture infected with type I herpes simplex virus (HSV-I) [1]. It was interesting to investigate the potentiating effect of PABA in combination with modified nucleosides on a sensitive and, for the first time, ACG-resistant HSV-I strain.

MATERIALS AND METHODS

Vero cell culture from Tissue Culture Department of the Institute of Virology was used. The culture was maintained in Eagle's medium manufactured by the Institute of Poliomyelitis (Moscow) with 10% fetal calf serum. Two HSV-I strains were used: highly sensitive to ACG and resistant to it. HSV-I strain L_2 was obtained from the collection of the Institute of Virology and cloned in our laboratory. ACG-resistant HSV-I strain was prepared by serial passages of the initial HSV-I in the presence of ascending concentrations of ACG [7]. Eagle's medium with 5% fetal calf serum was the maintenance medium. The resultant resistant strain was denoted as HSV-I/ACG(R). The cytotoxicity of PABA in Vero cell culture was assessed by visual examination under light microscope using ascending concentrations of PABA; the effect of PABA on the intact cell morphology was evaluated after a 3-day incubation and compared with the morphology of control cell structures [5]. The cytotoxicity of the studied compounds was assessed in Vero cells in the concentrations used in studies of their antiviral effect and in higher concentrations. ACG, Zovirax (Wellcome), 5-(2-bromovinyl)-2'-deoxyuridine (provided by Dr. I. K. Mikhailopulo, Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Minsk), adenine 9-β-D-arabinofuranoside (Calbiochem), trisodium salt phosphonoacetate (provided by Dr. V. L. Florent'ev, V. A. Engel'gardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow), and ribavirin (virazole, ICN Pharmaceutical) were used.

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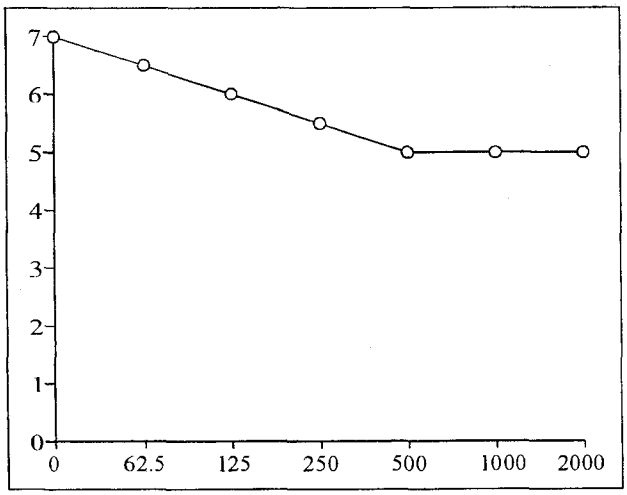

Fig. 1. Virucidal effect of para-aminobenzoic acid towards type I herpes simplex virus resistant to acycloguanosine in cell-free virus material. Ordinate: infectious titer of the virus in lg $TCID_{50}$/ml. Abscissa: concentrations of para-aminobenzoic acid, μg/ml. 120-min incubation at 37°C.

The virucidal effect of PABA on ACG-resistant HSV-I strain was assessed by incubation of cell-free virus-containing material followed by virus titration in experimental and control specimens. Antiviral effects of the studied compounds (multiplicity of infection 0.01 $TCID_{50}$/cell — tissue cytotoxic infective dose) were assessed from their capacity to prevent the development of virus-induced cytopathic effect by 50 and 95%, which was expressed as ED_{50} and ED_{95}, respectively [5]. Linbro 96-well plastic plates were used. In addition, combined antiviral action was characterized as the index of fraction inhibiting concentrations calculated from the conventional formula [4]. According to this formula, the indices of no more than 0.5 indicate a strong synergic effect, whereas indices of 0.9-0.5 point to poor synergism. All experiments were repeated three times.

RESULTS

PABA in a dose of up to 2000 μg/ml did not induce any appreciable cytotoxic effect on intact Vero cells after a 72-h incubation and did not increase the count of dead cells, as shown by trypan blue staining. None of the other studied agents exhibited cytotoxic activity, as shown by the two above-mentioned methods after a 96-h incubation with intact cells, which agrees with published data [5,10].

The sensitivity of the ACG-resistant HSV-I strain to ACG was 120-180 times lower than that of the initial strain: ED_{50} 60-90 μg/ml *vs.* 0.45 μg/ml. This HSV-I strain can be regarded as a thymidine kinase-defective strain or a strain with thymidine kinase with changed substrate specificity. PABA exhibited virucidal activity towards HSV-I strain highly sensitive to ACG [1]. Our experiments showed that PABA had the same effect on HSV-I strain resistant to ACG, i.e., on HSV-I/ACG (Fig. 1).

PABA in concentrations of 0-25 μg/ml did not prevent the virus-induced cytopathic effect and therefore did not inhibit the reproduction of the two HSV-I strains.

Table 1 shows that PABA stimulates the antiherpetic effect of modified nucleosides even in the concentrations which do not suppress the virus cytopathic action in the absence of PABA. Combined use of PABA with ACG and 5-(2-bromovinyl)-2'-deoxyuridine led to a 8-fold decrease in ED_{50} and ED_{95}, and PABA combination with adenine 9-β-D-arabinofuranoside decreased its concentration 5.5 times. These results indicate that antiviral effect of PABA combination with ACG is synergic, i.e., PABA alone possesses no antiviral effect.

Study of tentative stimulating antiherpetic effect of PABA on ACG-resistant HSV-I strain was of

TABLE 1. Stimulation of Antiherpetic Effects of Modified Nucleosides by PABA in Cell Culture

Compound	ED50, μg/ml	ED95, μg/ml	Index of fraction inhibiting concentrations
ACG	0.45	0.90	
ACG+PABA	0.055	0.112	0.11
5-(2-Bromovinyl)-2'-deoxyuridine	0.006	0.012	
5-(2-Bromovinyl)-2'-deoxyuridine+PABA	0.0007	0.0015	0.25
Adenine 9-β-d-arabinofuranoside	7.5	15.0	
Adenine 9-β-d-arabinofuranoside+PABA	1.87	3.75	
Adenine 9-β-d-arabinofuranoside*	7.5	15.0	
Adenine 9-β-d-arabinofuranoside+PABA*	1.87	3.75	0.4

Note. Multiplicity of infection 0.01 TCID50/cell. Results recorded 48 h postinfection. PABA concentration 0.035 μg/ml. *ACG-resistant HSV-I strain. Nucleosides alone possess no antiviral activity in concentrations 2, 4, and more times lower than those corresponding to their ED50 values. Mean values of 3 experiments are presented.

special interest. Adenine 9-β-D-arabinofuranoside suppressed the reproduction of ACG-resistant HSV-I strain as effectively as that of the ACG-sensitive strain, and its effect was also stimulated by PABA (Table 1). Investigation of PABA stimulation of the antiherpetic effect of phosphonoacetic acid showed a decrease in ED_{50} in the presence of PABA from 12.5 to 3.1 mg/ml. The effect was observed in the presence of 0.75-12.5 mg/ml PABA (the results were obtained in experiments performed together with Dr. V. M. Shobukhov).

PABA stimulates antiherpetic effects of modified nucleosides in concentrations of 0.017-6 μg/ml; the effect is highly reproducible. These results open new prospects in the therapy of herpetic infection, specifically, in cases when ACG is ineffective. No synergic effect of PABA was observed with ribavirin in the same model.

The revealed combined antiherpetic effect can be regarded as a new one, because the synergic antiherpetic effect of PABA combinations with ACG, 5-(2-bromovinyl)-2'-deoxyuridine, and adenine 9-β-D-arabinofuranoside differs from the known principle of combined drug therapy in this model: PABA alone displays no antiherpetic activity. Its activating antiviral activity in the above combinations manifests itself when the production of viral DNA is inhibited, which is determined by the mechanisms of action of ACG, 5-(2-bromovinyl)-2'-deoxyuridine, adenine 9-β-D-arabinofuranoside, and even phosphonoacetic acid. Each of these compounds has different effects on the production of viral DNA, eventually inhibiting the activity of viral DNA-polymerase. We think that the effect of PABA can be confined to this stage of HSV-I reproduction. There are virtually no facts explaining PABA potentiation of antiviral effects of the studied agents, but there is evidence that PABA contributes to stimulation of reparative processes of bacterial DNA induced by chemical mutagens [2,3]. Therefore, it can be suggested that PABA can modify the function of DNA-polymerase. It is noteworthy that PABA does not increase antiherpetic effect of the nucleoside ribavirine whose antiviral effect is not due to primary inhibition of the production of viral DNA.

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