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COMBINED ANTIINFLUENZA ACTIVITY OF A PLANT PREPARATION SHS-174 AND AMANTADINE DERIVATIVES

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Summary. – The results of the study on the combined antiviral activity of the SHS-174 preparation (a lyophylized infusion from three higher plants) and three amantadine derivatives (rimantadine, amantadine glucuronide and its derivative) are presented. The antiviral effect of the drugs on the reproduction of influenza virus strains A/H1N1 and A/H3N2 *in vitro* was studied. The combined antiviral effect was evaluated on the basis of viral yields and in many cases a synergism was found. The most synergistic effect was shown for the combination of SHS-174 with the derivative of amantadine glucuronide.

Key words: antiviral activity; influenza virus; plant preparation SHS-174; amantadine derivatives; combined effect

Introduction

The combined use of viral inhibitors is a promising approach for increasing the effectiveness of antiviral chemotherapy. It allows reduction of doses of individual agents and possible dose-related toxicity, potentiation of antiviral activity and prevention of the emergency of drug resistence. According to literature data it is promising to combine also potential antiviral substances of natural and synthetic origin (Dzeguze *et al.*, 1982; Musci, 1984; Uzunov *et al.*, 1991).

This paper presents the results from the combined application of the plant preparation SHS-174 and three amantadine derivatives on the influenza virus-cell culture system. Preliminary data of a part of this study were already published (Serkedjieva and Zgorniak-Nowosielska, 1991).

Materials and Methods

Drugs. The SHS-174 preparation is a lyophilized infusion from flowers of *Sambucus nigra L.*, aerial parts of *Hypericum perforatum L.* and roots of *Saponaria officinalis L.* It was provided by Dr. J. Grzybek, Department of Pharmaceutical Botany, Medical Academy, Krakow. The preparation was dissolved in sterile distilled water and used in non-toxic concentrations.

Amantadine derivatives: Gludantine (amantadine glucuronide, G1) and its derivative (dG1) were provided by Dr. M. Lidaks from the Institute of Organic Synthesis, Latvian Academy of Sciences, Riga. Rimantadine (alpha-methyl-1-amino-adamantanemethylamine hydrochloride, R) was a gift from Dr. M. Indulen from the Institute of Microbiology, Latvian Academy of Sciences, Riga. The substances were used as water solutions in non-toxic concentrations.

Viruses. Influenza virus strains A/PR/8 (H1N1) and A/Hong Kong/1/68 (H3N2) were grown in embryonated eggs and used as allantoic fluids.

Tissue cultures of chlorioallantoic membranes (CAM) were prepared by standard procedure.

Antiviral activity. The substances SHS-174, G1, dG1, R, and their combinations were applied simultaneously with virus inoculum. Virus titrations of media of cultures with or without antiviral drugs were carried out 48 hr post infection at 37 °C. The infectious titers were expressed in log ID_{50}/ml. The antiviral effect of the substances was determined by the difference of the infectious titers presented as mean values from 3–6 experiments. Significant was considered a reduction of virus yield ≥ 1 log.

The type of the combined antiviral effect was determined according to Schinazi *et al.* (1982), on the basis of virus yields. The fractional yield of the compound A (Y_A) was defined as the virus titer obtained in the presence of the compound. Similar definitions were used for the compound B (Y_B) and their combination (Y_{AB}). Then Y_C could be calculated using the equation:

$$Y_C = Y_A \times Y_B$$

Then if $Y_C > Y_{AB}$ - the effect was synergistic, if $Y_C = Y_{AB}$ - the effect was additive, and if $Y_C < Y_{AB}$ - the effect was subadditive.

Results and Discussion

The maximal tolerated concentrations (MTC) of SHS-174, G1, dG1 and R for CAM cultures are presented in Table 1. From dose - response dependence

Table 1. Properties of the compounds SHS-174, G1, dG1 and R in relation to influenza viruses A/H1N1 and A/H3N2 and CAM cultures

	Maximal tolerated concentration (MTC) μg/ml			
	SHS-174	G1	dG1	R
CAM culture	1500	6000	3000	600
	Minimal inhibitory concentration (MIC) μg/ml			
	SHS-174	G1	dG1	R
A/H1N1	260.0	270.0	62.5	10.6
A/H3N2	125.0	190.0	50.0	9.2
	Selectivity index (SI = MTC/MIC)			
	SHS-174	G1	dG1	R
A/H1N1	5.8	22.2	48	58.3
A/H3N2	12	31.6	60	65.2

curves the minimal inhibitory concentrations (MIC) of these compounds for influenza viruses A/H1N1 and A/H3N2 in CAM cultures were found and the selectivity indices (SI) were calculated (Table 1).

The results of the combined use of SHS-174 with G1, dG1 or R on the reproduction of influenza viruses A/H1N1 and A/H3N2 in CAM cultures are presented in Fig. 1 - Fig. 6.

Usually the combinations had a more pronounced inhibitory effect than the individual substances. Many combinations proved to be synergistic (Table 2). Most effective, all but one synergistic, were the combinations of SHS-174 with dG1 against A/H3N2.

The combined effect of SHS-174 with dG1 against A/H1N1 was usually subadditive but one additive and three synergistic combinations were found as well.

The effects of the combinations of SHS-174 with G1 and SHS-174 with dG1 were diverse. While the combinations of SHS-174 with dG1 often resulted in synergistic inhibitory effect, the simultaneous use of SHS-174 and G1 usually yielded a subadditive effect. The combination of SHS-174 with R was often subadditive, however, four synergistic and four additive combinations were found as well.

The combined use of the plant preparation SHS-174 with amantadine derivatives usually resulted in increased inhibition of virus reproduction. As useful could be considered combinations which had at least an additive inhibitory effect (Schinazi *et al.*, 1982). The combinations of SHS-174 with dG1 were most successful in this respect.

The results from the study of the inhibitory effect of the combination of SHS-174 with R showed that it was very important to pay much attention to the choice of appropriate doses of the different substances in order to evaluate synergistic antiviral effects.

Earlier it was found that the SHS-174 plant preparation affected the reproduction of different influenza virus strains *in vitro* and *in vivo* and the propagation of herpes simplex virus *in vitro* (Serkedjieva *et al.* 1990). The effect was dose-dependent, it was not due to a virocidal activity and reached maximal values when the substance was added after virus adsorption.

The chemical analysis of the SHS-174 preparation, performed by thin layer chromatography (Zawilinska *et al.*, 1990) revealed the presence of flavonoids, triterpene saponins, phenolic acids, tannins and polysaccharides. These chemical compounds could be responsible for the antiviral properties of the plant preparation.

Rimantadine is a highly effective drug in the prophylaxis and treatment of influenza A virus infection (Wingfield *et al.*, 1969). With many influenza virus strains the inhibition occurs at an early stage of virus infection, preventing virus uncoating (Skehel *et al.*, 1982). In certain influenza H7 infections the inhibition occurs at a later stage of replication and prevents the virus release by blocking of an effect on M2 protein and M2 mediated alteration of haemagglutinin (Hay, 1989).

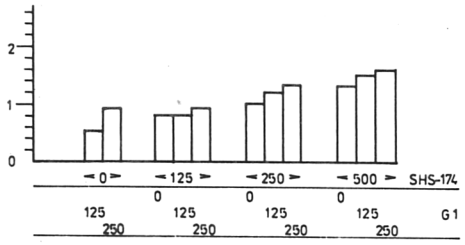

Fig. 1
Combined inhibitory effect of SHS-174 with G1 on the reproduction of influenza virus A/H1N1 in CAM cultures
Ordinate: reduction of virus titer by the substance(s) in log ID_{50}/ml; abscissa: μg/ml.

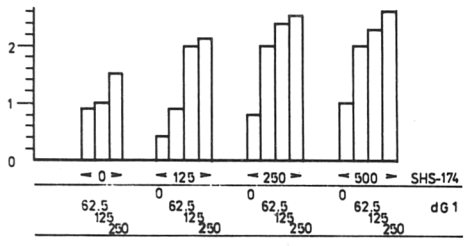

Fig. 2
Combined inhibitory effect of SHS-174 with dG1 on the reproduction of influenza virus A/H1N1 in CAM cultures
For legend see Fig. 1.

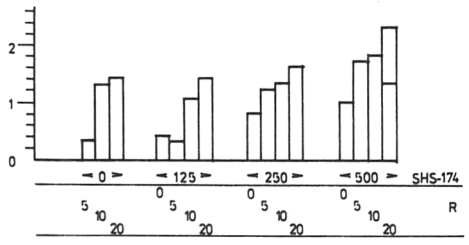

Fig. 3
Combined inhibitory effect of SHS-174 with R on the reproduction of influenza virus A/H1N1 in CAM cultures
For legend see Fig. 1.

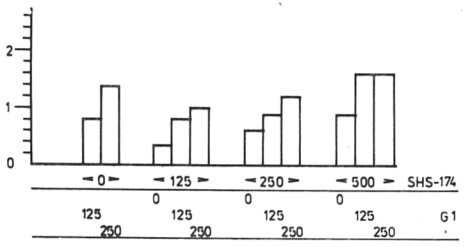

Fig. 4
Combined inhibitory effect of SHS-174 with G1 on the reproduction of influenza virus 1/H3N2 in CAM cultures
For legend see Fig. 1.

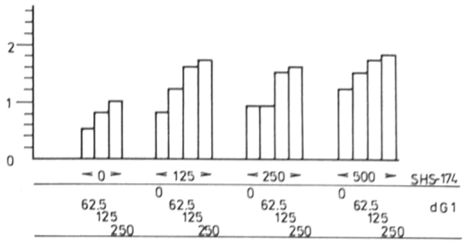

Fig. 5
Combined inhibitory effect of SHS-174 with dG1 on the reproduction of influenza virus A/H3N2 in CAM cultures
For legend see Fig. 1.

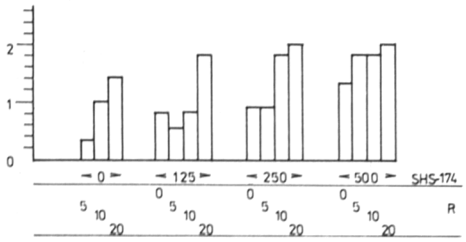

Fig. 6
Combined inhibitory effect of SHS-174 with R on the reproduction of influenza virus A/H3N2 in CAM cultures
For legend see Fig. 1.

Table 2. Synergistic inhibitory effect of SHS-174 with dG1 and R on influenza virus reproduction in CAM cultures

	SHS-174	dG1	R	Y_{AB}	Y_C
	μg/ml				
A/H1N1	250	125		0.74	0.77
	125	250		0.68	0.69
	125	125		0.7	0.72
	500		5	0.66	0.7
	250		10	0.66	0.67
A/H3N2	250	250		0.54	0.6
	250	125		0.6	0.7
	250	62.5		0.65	0.67
	125	250		0.56	0.64
	125	125		0.58	0.72
	125	62.5		0.65	0.7
	62.5	250		0.65	0.69
	62.5	125		0.65	0.78
	250		20	0.6	0.62
	250		5	0.53	0.75

Indulen *et al.* (1973) studied the antiinfluenza action of amantadine glucuronide. The substance was less toxic to CAM than amantadine and inhibited all tested viral strains of type A. No data were presented on its mechanism of action. No published data are available on the properties or the biological activities of amantadine glucuronide used in our experiments.

At the present stage of our investigation the mechanism of the combined antiviral effect of the SHS-174 plant preparation with the amantadine derivatives cannot be seriously discussed yet.

Previously we have studied the combined antiviral effect of another viral inhibitor of natural origin - the polyphenolic complex (PC) isolated from the medicinal plant *Geranium sanguineum L.* - with rimantadine and amantadine derivatives on the reproduction of influenza A virus (Indulen *et al.*, 1987; Serkedjieva *et al.* 1986; Uzunov *et al.* 1991). We have found synergistic combinations of PC and R *in vitro, in ovo,* and *in vivo.* The simultaneous use of PC with G1 or dG1 resulted in synergistic inhibitory effect in all combinations.

The inhibitory activity of antiviral agents of plant origin depends on their chemical composition. Obviously their composition is important also for the expression of combined inhibitory effects.

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