Inhibition of the Growth of Hemadsorption 2 Virus by Three Acyl Derivatives of Amino Acids

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Three acyl derivatives of amino acids, dicarbobenzyloxyl-lysine sodium, carbobenzyloxyl-aspartic acid-β-benzyl ester potassium, and N-3-phenylpropionyl-S-benzyl-L-cysteine potassium inhibit the growth of parainfluenza 1 (hemadsorption 2) virus. The growth of simian virus 40, vaccinia, poliomyelitis type 1, Semliki Forest, Eastern equine encephalitis, and Western equine encephalitis viruses was not affected by these compounds. Four other acyl derivatives of amino acids did not inhibit the growth of any of the viruses tested.

Carbobenzoxy, phenylacetyl, and phenylpropionyl derivatives of amino acids were found to markedly inhibit the growth of Ehrlich ascites tumor in mice (7, 8; Schlesinger, Grossowicz, and Lichtenstein, submitted for publication). Such derivatives also strongly inhibit a number of enzymatic systems: phenylalanine incorporation in a cell free system from ascites cells (1), rat liver asparaginase and glutaminase (4, 5), rat liver and ovine brain glutamine synthetase (7, 8), rat liver nicotinamide adenine dinucleotide synthetase (6), and the purine synthesizing system of pigeon and chicken liver (3).

Since the growth of animal viruses is dependent on enzyme systems of the host-cell and few of these systems are known to be inhibited by several acyl derivatives of amino acids, we asked whether the growth of animal viruses is affected when the cells are treated with such compounds. Vaccinia WR strain, herpes simplex type 1, poliomyelitis type 1, simian virus 40, and parainfluenza 1 (hemadsorption [HA] 2) viruses were grown in BSC1 cells, whereas Semliki Forest, Eastern equine encephalitis, and Western equine encephalitis viruses were grown in chicken fibroblasts.

The compounds which we tested for antiviral activity were: N-carbobenzoxy-S-benzyl-L-cysteine sodium (DWK 1), carbobenzoxy-L-glutamic acid-γ-benzyl ester sodium (DWK 5), dicarbobenzoxy-L-lysine sodium (DWK 6), carbobenzoxy-L-aspartic acid-β-benzyl ester potassium (DWK 7), N-3-phenylpropionyl-S-benzyl-L-cysteine potassium (DWK 19), di-3-phenylpropionyl-L-cystine dipotassium (DWK 21), and phenylacetyl-D-phenylalanine potassium (DWK 22). Since the toxicity of these compounds to cells in culture varies from one compound to another, the highest concentration which does not cause a visible cell degeneration was used.

Three compounds: DWK 6 (5 mM), DWK 7 (10 mM), and DWK 19 (5 mM) inhibited the growth of HA 2 virus, a member of parainfluenza 1 viruses, in BSC1 cells (Fig. 1). In untreated culture, virus infectivity increased by almost 1.5 log10 mean tissue culture infective doses within the first day after infection. In the three treated cultures the infectivity decreased rapidly during this period of time and started to rise during the second day. In DWK 6- and DWK 7-treated cultures, virus infectivity leveled off between days 2 and 3; however, in DWK 19-treated culture, the virus titer increased and reached almost the level of the control culture at the end of the third day after infection.

In parallel to the growth of HA 2 virus, the growth of SV40 virus was tested (Fig. 1). In comparison to the antiviral effect of the three compounds on HA 2 virus, the growth of SV40 in BSC1 cells was not affected by these compounds. This suggests that the effect of the three drugs on HA 2 virus is specific and does not result from unsselective toxicity of the compounds to the host cell.

We examined whether addition of the three compounds to the cultures at different times, with regard to infection with HA 2 virus, changes the antiviral effect. A similar rate of inhibition was observed when the compounds were added 2 h before infection, at the time of infection, or 2 or 4 h afterwards. The possibility of a direct effect of the compounds on HA 2 virus was tested by incubating the virus with the compounds at a final concentration of 25 mM for 60 min at 37°C followed by titration of the virus in BSC1 cells. No decrease in virus...
infectivity was detected. This finding suggests that the three drugs DWK 6, DWK 7, and DWK 19 affect a step associated with virus growth.

Four other compounds, DWK 1 (1.25 mM), DWK 5 (2.5 mM), DWK 21 (25 mM), and DWK 22 (25 mM), did not significantly inhibit the growth of HA 2 virus. In addition to examination of the effect of the seven compounds on HA 2 virus and SV40, we tested their effect on the growth of vaccinia, poliomyelitis type 1, and herpes viruses in BSC1 cells and of Semliki Forest, Eastern equine encephalitis, and Western equine encephalitis viruses in chicken fibroblasts. These acyl derivatives of amino acids did not inhibit the growth of the six viruses.

The composition of the active site of the acyl derivatives of amino acids DWK 6, DWK 7, and DWK 19, which determines the antiviral activity for HA 2 virus, is not clear yet. These active compounds are either carbobenzoxy or phenylpropionyl derivatives of lysine, cysteine, and aspartic acid. A further study would provide additional information concerning the structure of the molecule which is needed for the antiviral activity and the mode of action of these drugs.

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**LITERATURE CITED**


