Studies on Antiviral Glycosides. 4. Inhibition of the Multiplication of Paramyxoviruses by Phenyl-6-chloro-6-deoxy-\(\beta\)-D-glucopyranoside

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SUMMARY

The antiviral activity of phenyl-6-chloro-6-deoxy-\(\beta\)-D-glucopyranoside (PCG) was studied. PCG specifically inhibited the growth of paramyxoviruses including Sendai, measles and Newcastle disease viruses in LLCMK₂ cells at a concentration of 0.5 to 1.0 mM, but did not restrict the multiplication of other RNA viruses (influenza, vesicular stomatitis and polio viruses) at these concentrations.

PCG might act in the late stage during virus replication of Sendai virus as it did not inhibit virus RNA and protein synthesis in the infected cells. Comparative studies on the biological properties of virus particles grown in the presence and absence of PCG demonstrated that treatment with it caused the formation of non-haemagglutinating particles.

Antiviral activities of sugar derivatives have been reported by many investigators. Evidence indicated that 2-deoxy-D-glucose (2-dG) inhibited the multiplication of several enveloped viruses by interfering with the process of virus glycosylation as an anti-metabolite of mannose rather than glucose. Glucosamine had two different inhibitory mechanisms, in blocking the maturation of virus glycoproteins such as 2-dG and in inhibiting virus RNA synthesis by reducing the UTP pool in the infected cells (for review, see Scholtissek, 1975). The fluorosugars, 2-deoxy-2-fluoro-D-glucose and 2-deoxy-2-fluoro-D-mannose, were also found to have the same antiviral mechanism as 2-dG with much higher activities (Schmidt et al. 1976) and 6-amino-6-deoxy-D-glucose has been reported to be an interferon inducer having a prophylactic effect against influenza virus-infected mice (Hruskova et al. 1975).

We have previously reported the antiviral activities of a series of phenyl glucoside derivatives newly synthesized in our laboratory and the relationship between their structures and activities (Arita et al. 1978). In this study, we found that phenyl-6-chloro-6-deoxy-\(\beta\)-D-glucopyranoside (PCG) specifically inhibited the production of infectious paramyxoviruses, but not of myxoviruses and other enveloped and non-enveloped RNA viruses, indicating that it has a different antiviral mechanism from 2-dG and glucosamine.

To examine antiviral activity of the compound against the replication of the virus, virus growth inhibition tests were essentially done by the method described previously (Sato et al. 1977). LLCMK₂ cell monolayers grown for 3 to 4 days were infected with viruses at a multiplicity of 1 to 10 p.f.u. After adsorption of virus, the infected cells were fed with Eagle’s minimal essential medium (MEM) with or without PCG (1 mM) and incubated at 37 °C until the maximum virus yield in a one-step growth cycle was reached in the untreated culture. After freezing and thawing the harvested culture fluids, virus titration was performed by plaque assays with the aid of trypsin for Sendai and measles viruses as described previously (Sugita et al. 1974) and without trypsin for vesicular stomatitis (VSV) and polioviruses. Haemagglutinating activity (HA) was measured for Newcastle disease virus (NDV) and influenza virus using chicken erythrocytes.

We found in this experiment that yields of paramyxoviruses, such as Sendai, measles
Table 1. Effect of PCG on the production of haemagglutinin and infectious and physical particles in Sendai virus-infected cell cultures

<table>
<thead>
<tr>
<th>Drug concn (mM)</th>
<th>Infectivity* (p.f.u./ml)</th>
<th>Haemagglutinin* (HAU/ml)</th>
<th>Total ct/min virus particles†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.0 × 10³ (1.0)</td>
<td>4.0 (0.8)</td>
<td>4620 (19)</td>
</tr>
<tr>
<td>1</td>
<td>2.4 × 10³ (1.2)</td>
<td>16 (3.2)</td>
<td>10360 (36)</td>
</tr>
<tr>
<td>0.5</td>
<td>1.4 × 10³ (7.0)</td>
<td>64 (12.5)</td>
<td>15410 (42)</td>
</tr>
<tr>
<td>0</td>
<td>2.0 × 10¹ (10.0)</td>
<td>572 (100)</td>
<td>34560 (100)</td>
</tr>
</tbody>
</table>

* These activities were measured in the infected cell cultures at 24 h after virus infection. HAU was measured according to Salk's pattern method (Salk, 1944).
† Infected cell monolayers were labelled with ³H-leucine (300 #Ci/ml) during 1 to 24 h p.i. The cultures were frozen and thawed twice and purified by two cycles of differential centrifugation (5000 rev/min for 15 min and 20000 rev/min for 30 min in a Hitachi RP-40A rotor). The pellets were suspended in 0.4 ml phosphate-buffered saline free of calcium and magnesium. The radioactivity of 50 μl amounts was measured in a Packard liquid scintillation counter.
‡ Numbers in parentheses are percentages of the control.

Table 2. Comparison of biological properties of Sendai virions grown in the presence or absence of PCG in LLCMK₂ cells

<table>
<thead>
<tr>
<th>Virion</th>
<th>Biological activities*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAU</td>
</tr>
<tr>
<td>Control</td>
<td>23814</td>
</tr>
<tr>
<td>PCG-treated</td>
<td>1684 (7.1)†</td>
</tr>
</tbody>
</table>

* The biological activities are expressed as units/mg protein of Sendai virions. Protein assay was performed by Lowry's method (Lowry et al. 1951). NAU was measured according to the method described by Maeno et al. (1970). HLU was determined after treatment with trypsin (Shimizu et al. 1976).
† Numbers in parentheses are percentages of control viron activities.

and NDV, were reduced to less than 25 % by PCG, while those of other viruses including influenza (A₁, A₂ and B), polio and VSV were not (data not shown). Sendai virus was the most sensitive and the virus yields of all four strains examined, Fushimi, Z, RL and RS, in PCG containing cultures were about 1 % of the control. One of them, Fushimi strain, was used for further studies.

Table 1 shows the dose effect of PCG on the production of haemagglutinin and infectious and physical particles. The number of physical particles was estimated by determining the radioactivity of labelled virions purified by two cycles of differential centrifugation from the infected cells with or without PCG. The results indicate that the haemagglutinating activity and infectivity produced by the treated cells were more strongly reduced than the amount of sedimentable virus, suggesting that PCG caused the production of non-haemagglutinating particles. To reach this conclusion, we compared the biological activities of virus particles purified from infected cells treated with or without PCG (Table 2). HA, neuraminidase (NA), haemolytic (HL) activities and infectivity per mg virus protein of virus particles produced in the presence of 1 mM-PCG were 7.1, 8.5, 6.7 and 1.1 % respectively of those of control virus particles. From these results, it appears that PCG caused the formation of virus particles without the activities of the virus envelope glycoprotein molecules (Chen et al. 1971). The fact that these activities were reduced to the same extent by PCG would indicate that the inhibitor might act on the biosynthesis of functional HANA protein which is the glycoprotein having both HA and NA activities in Sendai virus (Tozawa et al. 1973). The loss of HL could be a secondary effect because the haemolytic reaction only occurs after virus adsorption to red blood cells (Sokol & Neurath, 1962).
has been reported that the electrophoretic mobility of the HA polypeptide of influenza virus treated with 2-dG and glucosamine progressively increased (Schwarz & Klenk, 1974; Nakamura & Compans, 1978). However, we could not find any difference in the electrophoretic mobility of the HANA proteins of virions produced with or without PCG in SDS–polyacrylamide gel electrophoresis (Maizel, 1969). However, it is possible that PCG blocks the glycosylation of virus glycoproteins in such a way that their electrophoretic mobilities do not change. A study to examine this possibility is now in progress. It is noteworthy that PCG preferentially inhibited the growth of paramyxoviruses, in contrast to 2-dG and glucosamine. Another compound which has been reported to have selective inhibitory action against paramyxoviruses is cordycepin, whose action is to block the synthesis of poly(A) sequences contained in the mRNAs of paramyxoviruses, but not of myxoviruses (Mahy et al. 1973). However, PCG did not inhibit virus RNA synthesis in Sendai virus-infected cells, nor did it inhibit virus protein synthesis (data not shown).

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REFERENCES


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