CHAPTER 8

INHIBITION OF BOVINE HERPESVIRUS 4 REPLICATION IN VITRO BY SELECTED ANTIVIRAL AGENTS

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Abstract

In this study the susceptibility of bovine herpesvirus 4 (BHV4) to various antiviral agents was evaluated. Brivudin inhibited the replication of BHV4 in bovine umbilical cord endothelial cells with a 50% effective concentration (EC50) value of 0.05 µg/ml. Cidofovir and ganciclovir also inhibited the replication of BHV4, but with EC50-values of 0.2 – 0.8 µg/ml and 6.3 – 12.5 µg/ml, respectively. Inhibitory activity on the replication of BHV4 was observed for foscarnet at a concentration of 100 µg/ml, whereas no inhibition of BHV4 replication was noted for acyclovir, adeovir, and penciclovir at concentrations of ≤100 µg/ml. Brivudin and cidofovir, and to a lesser extent ganciclovir, may have potential for the treatment of BHV4 infections.
1. Introduction

Bovine herpesvirus 4 (BHV4), one of the members of the *Gammaherpesvirinae* subfamily, has been isolated from cows with various diseases e.g. mammary pustular dermatitis (Reed et al., 1977). Recently, BHV4 has been isolated from milk samples of cows with clinical mastitis, and not from milk samples of control cows (Wellenberg et al., 2000). Following a simultaneous intramammary and intranasal inoculation of lactating cows with BHV4, replication of BHV4 in the mammary gland and an increase in milk somatic cell counts were noted (Wellenberg et al., 2001). Zadoks et al. (submitted) reported an association between BHV4 seropositivity and the occurrence of bovine mastitis caused by *Staphylococcus aureus*. These reports suggest that BHV4 plays a role in the aetiology of bovine mastitis. A compound that would specifically inhibit the replication of BHV4 may have potential for the treatment of BHV4 infections.

The susceptibility of murine gammaherpesvirus 68 (MHV-68), another member of the subfamily of the *Gammaherpesvirinae*, to antiviral agents has been reported by Sunil-Chandra et al. (1994a; 1994b), and Neyts and De Clercq (1998). There have been no reports on the susceptibility of BHV4 to antiviral agents. Such knowledge may be of interest for the treatment of BHV4 infections, and also provide deeper insight into the mode of replication of this virus. Therefore, we examined the susceptibility of BHV4 to selected compounds with proven anti-herpesvirus activity.
2. Materials and methods

2.1 Virus and cells

The Dutch BHV4 strain Tolakker has been isolated from a cow with signs of clinical mastitis (Wellenberg et al., 2000). Bovine umbilical cord endothelial (BUE) cells were used for BHV4 propagation. BUE cells were grown and maintained in Dulbecco’s minimal essential medium (DMEM) (Gibco Laboratories, Life Technologies Inc., USA) supplemented with 2% foetal bovine serum (Gibco Laboratories, Life Technologies Inc., USA). The following antiviral agents were used; penciclovir (PCV), ganciclovir (DHPG), acyclovir (ACV), cidofovir (HPMPC), adefovir (PMEA), foscarnet (PFA), and brivudin (BVDU). Antiviral activity was studied on semi-confluent monolayers of BUE cells grown in 96-well cell culture plates (Costar). Cells were inoculated with BHV4 at a multiplicity of infection (m.o.i.) of 0.1. Following an adsorption period at 37°C for 2 hours, BUE cell cultures were washed with phosphate buffered saline solution (PBS).

2.2 Antiviral agents

Serial two-fold dilutions of each antiviral agent, ranging from 0.05 µg/ml to 100 µg/ml, were prepared in Earle’s minimal essential medium (EMEM + 2% normal calf serum) using dummy plates. A volume of 100 µl of each dilution was added in duplicate to the washed BUE cell cultures, and cell cultures were incubated at 37°C for 7 days (5% CO₂). Each serial dilution of the antiviral agents was checked for BUE cell toxicity by adding a volume of 100 µl of each dilution (in duplicate) to semi-confluent monolayers of BUE cells that had not been inoculated with BHV4. For each antiviral agent, also a cell control and a virus-growth control was incorporated into each cell culture plate. Therefore, non-infected semi-confluent monolayers of BUE cells, with the addition of 100 µl EMEM (+ 2% normal calf serum), but without the antiviral agents, were used as cell control. Semi-confluent monolayers of BUE cells that were infected with BHV4 (m.o.i. = 0.1) were used as virus-growth control. The virus-growth control was incubated and washed as described above. After the wash procedure 100 µl EMEM (+ 2% normal calf serum), but without the antiviral agents, was added to the wells. BUE cell toxicity, cell and virus-growth controls, were incubated for 7 days at 37°C (5% CO₂). The appearance and degree of cytopathic effects (cpe) in each well was recorded microscopically at day 7 post-infection. The EC₅₀-value was calculated on the concentration that inhibited virus-induced cpe formation by 50%, and the complete inhibition (CI) of BHV4 replication was recorded in case no cpe was observed.
3. Results and discussion

The *in vitro* activities of the selected antiviral agents on the replication of BHV4 in BUE cell cultures are presented in Table 1. Complete inhibition of virus-induced cpe formation was observed in BHV4 infected BUE cells treated with BVDU at concentrations of \( \geq 0.05 \, \mu g/ml \) (EC\(_{50}\)-value: 0.05 µg/ml), with HPMPC at concentrations of \( \geq 1.6 \text{ – } 3.2 \, \mu g/ml \) (EC\(_{50}\)-values: 0.2 – 0.8 µg/ml), and with DHPG at concentrations of \( \geq 25 \, \mu g/ml \) (EC\(_{50}\)-values: 6.3 – 12.5 µg/ml). A limited inhibition of virus-induced cpe formation was observed in BHV4 infected BUE cells treated with PFA at a concentration of 100 µg/ml, and no inhibition was observed with ACV, PMEA or PCV. Complete destruction of the BUE cell monolayers (100% cpe) was observed in all wells used for virus-growth control, and no cpe was observed in all BUE cell control wells.

**Table 1.** Inhibitory effects of selected anti-herpesvirus agents on the in vitro replication of BHV4 (strain Tolakker).

<table>
<thead>
<tr>
<th>Antiviral agent</th>
<th>EC(_{50})(^a) (µg/ml)</th>
<th>CI(^b) (µg/ml)</th>
<th>MTC(^c) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDU</td>
<td>0.05</td>
<td>( \geq 0.05 )</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>HPMPC</td>
<td>0.2 – 0.8</td>
<td>( \geq 1.6 \text{ – } 3.2 )</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>DHPG</td>
<td>6.3 – 12.5</td>
<td>( \geq 25 )</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>PFA</td>
<td>100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>ACV</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>PCV</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>PMEA</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

\(^{a}\): concentration of antiviral agent required to reduce BHV4-induced cpe formation by 50%

\(^{b}\): concentration of antiviral agent required for complete inhibition of BHV4-induced cpe formation

\(^{c}\): minimal toxic concentration (concentration required to alter morphology of BUE cells)

BVDU, ACV, PCV and DHPG all depend on a virus-encoded thymidine kinase (TK) for activation. However, in this study only BVDU and DHPG inhibited BHV4 replication. Compared to MHV-68, BHV4 seems to be more susceptible to BVDU, whereas both viruses are equally susceptible to DHPG. HPMPC, which does not depend on the viral TK for activation, but probably on the DNA polymerisation process, proved to be a potent inhibitor of the replication of BHV4. Two other
gammaherpesviruses, i.e. MHV-68 and Epstein-Barr virus, proved also to be susceptible to HPMPC. Another acyclic nucleoside phosphonate, namely adefovir (PMEA), which has potent anti-retrovirus and anti-herpesvirus activities (Naesens et al., 1997) was devoid of anti-BHV4 activity.

In conclusion, this study provides information on the susceptibility of BHV4 to a selection of anti-herpesvirus agents. This information offers opportunities for designing strategies to inhibit BHV4 replication in e.g. the mammary gland or to treat pustular dermatitis.
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References


