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CORNEAL TISSUE CULTURE: A novel model for the study of activity of antiviral agents against adenovirus and HSV

J. Cinatl jr.[1], J.-U. Vogel[1], H. Guembel[2], J. Cinatl[1], M. Hacker[2], H.W. Doerr[1]
[1]Institute for Medical Virology, Paul-Ehrlich Str. 40, D-60596 Frankfurt/M., FRG, [2]University Clinics Frankfurt/M., Dep. of Ophthalmology, FRG

Purpose: Activities of antiviral agents vary considerably in *in vitro* assays depending on the cell type used. We established a corneal tissue culture (CTC) system as ocular model for testing antiviral agents against adenovirus and HSV. **Methods:** Corneas (obtained from human cadavers unusable for angioplasty) were infected with different strains of HSV type 1 (HSV-1) and adenovirus and incubated in IMDM medium supplemented with 2% fetal bovine serum. Virus titre was quantified in terms of the 50% tissue culture infective dose ($TCID_{50}$). Moreover virus replication was monitored by expression of virus specific mRNA using RT-PCR. Antiviral effects of acyclovir (ACV) and cidofovir (HPMPC) in corneal tissue culture were compared with those in monkey kidney Vero and human foreskin fibroblast (HFF) cell lines. **Results:** Laboratory strains of adenoviruses types 2, 5 and 7 replicated in CTC to relatively low titres (10^3 to 10^4 $TCID_{50}$ per cornea) while clinical ocular isolates replicated to significantly higher titres (10^5 to 10^6 $TCID_{50}$ per cornea). HSV-1 laboratory and clinical strains replicated to equivalent titres in CTC. HPMPC was more effective (up to 5-fold) against adenoviruses in CTC than in Vero or HFF cell lines. ACV was more effective (up to 8-fold) against HSV-1 in CTC than in Vero cells while similar antiviral activity was found in HFF. **Conclusion:** The results showed that CTC may be used as a model for testing of antiviral agents against adenoviruses and HSV-1 and that their effects in CTC can differ considerably from those observed in cell lines commonly used for antiviral testing.

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The Same Point Mutation Within Region II of the Herpes Simplex Virus Type 1 (HSV-1) DNA Polymerase Gene Confers Resistance to Foscarnet (PFA) and the Phosphonylmethoxyethyl (PME) Derivatives of Adenine (PMEA) and 2,6-Diaminopurine (PMEDAP)

G. Andrei, R. Snoeck, E. De Clercq, P. Fiten and G. Opdenakker
Rega Institute for Medical Research, K.U.Leuven, Leuven, Belgium

We have shown that PFA-resistant (PFA^r) strains of HSV-1 are resistant not only to PFA and phosphonoacetic acid (PAA) but also to PMEA and PMEDAP. Similarly, those strains that were obtained under the selective pressure of PMEA and PMEDAP ($PMEA^r$, $PMEDAP^r$) showed cross-resistance to PFA and PAA. To identify the nucleotide changes that occurred in the PFA^r, $PMEA^r$ and $PMEDAP^r$ strains, we compared the nucleotide sequences of the DNA polymerase genes of these strains with the nucleotide sequence of the wild-type strain KOS. Each strain was plaque-purified and viral DNA was prepared. A 3.4 kb *BamH1* fragment containing 87% of the HSV-1 DNA polymerase gene coding region was purified from each viral genome and further digested with *SacI*; the two resulting fragments were subcloned into pUC18 and propagated in *E.coli*. Plasmid DNA was isolated and the inserts were sequenced using the dideoxynucleoside chain termination method with T7 DNA polymerase and Taq DNA polymerase in an automated laser fluorescent DNA sequencer. pUC/M13 reverse and universal primers and oligonucleotide primers based on the wild-type KOS strain sequence were used. Sequence analysis of the DNA polymerase gene of either the PFA^r, $PMEA^r$ or $PMEDAP^r$ strain revealed a single nucleotide change which resulted in a serine to asparagine change at position 724 within the conserved region II of the DNA polymerase. This mutation has already been found in a PFA^r mutant by Gibss *et al.* (Proc. Natl. Acad. Sci. USA 85: 6672-6676, 1988). The serine at position 724 is highly conserved in all the herpesvirus DNA polymerases. Whether the DNA polymerase 724 Ser → Asn mutation also accounts for resistance of other herpesviruses to PMEA and PMEDAP remains to be determined.

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Selectability of Varicella-Zoster Virus Resistant to Acyclovir and Penciclovir K. Shiraki, M. Ida, H. Sato, M. Kurokawa, S. Kageyama. Department of Virology, Toyama Medical and Pharmaceutical University, Toyama, Japan

Acyclovir (ACV) and penciclovir (PCV) are the highly selective anti-varicella-zoster virus (VZV) agents and possess a similar mechanism of anti-VZV action. We have reported some differences in the inhibitory process of plaque formation. Selectability of VZV resistant to ACV and PCV was examined in their presence. A thousand plaque forming units of cell-free VZV (6 times plaque-purified Kawaguchi strain) were inoculated into human embryonic lung cells (25 cm^2 flask) and subcultured in the presence of 2, 4, and subsequently 6 μg/ml of ACV or PCV. After 3 weeks' incubation viruses resistant to the drug were isolated and characterized. ACV-resistant viruses were obtained but PCV-resistant virus was not in 5 independent experiments, indicating significant less selectability of PCV-resistant virus ($P<0.05$ by Fisher test). Seven ACV-resistant viruses isolated were resistant to PCV and sorivudine but sensitive to phosphonoaceteic acid, and failed to induce thymidine kinase (TK) activity in their infected cells. Thus the isolated ACV-resistant viruses were TK-deficient mutants. This study demonstrated that drug-resistant VZV was less selectable in PCV than in ACV *in vitro* and that the ACV-resistant viruses selected were all TK-deficient mutants. This implies the difference in the inhibitory action between ACV and PCV.

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The differential effects of famciclovir and valaciclovir on the establishment of latent infections in mice.

Thackray, A.M. & Field, H.J. Centre for Veterinary Science, Cambridge University, Madingley Road Cambridge, CB3 0ES, UK.

Previous studies have shown that the nucleoside analogue prodrugs famciclovir (FCV) and valaciclovir (VACV) are potent inhibitors of HSV in a murine model (ear pinna) for cutaneous herpes simplex with or without immunosuppression. Furthermore, when recovered animals that had been treated during the first few days of the acute infection, were tested for the presence of latency, differences between mice treated with the two compounds emerged. Latent virus could be reactivated from explanted ganglia of significantly fewer mice treated with FCV[1]. This was true for both HSV-1 and HSV-2. In these experiments the test employed for latency was a biological one; the ability of ganglion explants to yield infectious virus after 5 days incubation. A further experiment was carried out in which ear pinna-infected mice were treated commencing 1,2,3,4, or 5 days after virus inoculation. The compounds were administered by oral gavage at 50 mg/kg, b.i.d. terminating on day 10 p.i. The target tissues (ear, brainstem, ganglia) from groups of mice were tested by a variety of techniques during the acute stage of the infection and again 8 weeks later. Ganglia were analysed by incubation whole for 5 days as before; by prolonged incubation (60 days); by incubation following enzymatic disaggregation; by in-situ hybridisation; and by PCR. The results from this study provide a better understanding of the effects of the two drugs on HSV replication in neural tissues *in vivo* and the greater potential of FCV than VACV for preventing the establishment of latency.

[1]**J. Inf. Dis. 173, in press, 1996.**