

Short communication

Parapoxviruses are strongly inhibited in vitro by cidofovir

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Received 25 July 2000; accepted 11 September 2000

Abstract

Three parapoxviruses which cause orf or related diseases in humans and animals and the orthopoxvirus, vaccinia virus, were tested for their in vitro sensitivity to cidofovir. The 50% inhibitory concentration for the three parapoxviruses was between 0.21 and 0.27 µg/ml and for vaccinia was 1.32 µg/ml. The selectivity index varied from 198 to 264 for the parapoxviruses and was 42 for vaccinia virus. Virus yield assays confirmed the ability of cidofovir to reduce ortho- and parapoxvirus replication. The efficacy of cidofovir against parapoxviruses justifies its evaluation as a candidate drug for the treatment of parapoxvirus infections in humans and animals. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cidofovir; Parapoxvirus; Orf; Pseudocowpox; Milker's nodule

Parapoxviruses cause orf in sheep and goats, papular stomatitis and pseudocowpox in cattle and skin lesions in other animals including red deer, seals, squirrels, reindeer, musk ox and camels (Reid, 1998). Human infection from contact with domestic animals is a common occupational disease with infection rates up to 34% in at-risk farming communities (Buchan, 1996). As well as acquiring orf from sheep and goats and milker's nodule from cattle people can be unwittingly infected from other species (Falk, 1978;

Smith et al., 1991). In people the typical 'target' lesions occur most commonly on the hands but tumour-like nodules can occur on the face (Rogers et al., 1989; Bodnar et al., 1999). While many cases are self-limiting resolving within 6–8 weeks, complications include: prolonged resolution time up to 6 months, secondary bacterial infection, regional lymphadenopathy, lymphangitis, erythema multiforme and bullous pemphigoid (Falk, 1978; Yirrell et al., 1994; Murphy and Ralfs, 1996). Extensive and recurring lesions have been described also in those with burns received at the time of infection and in immunosuppressed patients which has resulted in the development of 'giant orf' and, in one case, to amputation of the

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affected finger (Reid, 1998; Degraeve et al., 1999). Treatment of difficult cases has necessitated excision or cryotherapy.

The acyclic nucleoside phosphonate analogue (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC) (Cidofovir) has potent and selective activity against a broad spectrum of DNA viruses including the orthopoxvirus, vaccinia virus (VV) (De Clercq, 1997, 1998). We have tested *in vitro* the activity of HPMPC against three parapoxviruses (PPV) and VV.

The three PPVs were orf-11 from Scotland, NZ-2 from New Zealand and milker's node (MN) (strain B074) from Germany. The VV was strain Lister. Their origin and growth on semi-continuous fetal lamb muscle (FLM) cells, established at Moredun Research Institute, has been described (Housawi et al., 1998). Cells grown in 96-well microplates were infected with 20 pfu virus/well. After 2 h of incubation at 37°C, the infected cells were replenished with 0.15 ml medium containing serial dilutions of cidofovir in the range 0.2–20 µg/ml. When plaques were clearly visible they were counted microscopically after staining with 0.1% crystal violet. The minimum antiviral concentration (IC₅₀) was expressed as the dose required to inhibit virus-induced plaque formation by 50%. Cytotoxicity measurements were based on the inhibition of newly confluent monolayers of FLM cells grown in 96-well plates. Washed monolayers were incubated with maintenance medium containing serial dilutions of cidofovir at 37°C for 4 days. The cells were then trypsinised and the cell numbers determined with a Coulter Counter. The 50% cytotoxic concentration (CC₅₀) is the concentration required to reduce cell numbers by 50% relative to untreated control cell numbers. The selectivity index is the ratio of CC₅₀ for cell growth to IC₅₀ for antiviral activity.

The IC₅₀ (*n* = 4) for orf-11, NZ-2, MN and VV were 0.27 ± 0.05 , 0.28 ± 0.07 , 0.21 ± 0.06 and 1.32 ± 0.47 µg/ml, respectively. The CC₅₀ (*n* = 4) for the FLM cells was 55.5 ± 41.3 giving selectivity indices for the four viruses of 206, 198, 264 and 42.

The effect of varying concentrations of cidofovir on yields of the four viruses was also measured. Confluent FLM monolayers grown in six-well plates were infected at a moi of approximately 0.1. After 2 h incubation residual virus was removed and replaced by medium containing different concentrations of cidofovir. At daily intervals post-infection aliquots of supernatant were withdrawn and frozen at –70°C until they were titrated for virus in 96-well microplates (Fig. 1). All concentrations of cidofovir reduced virus yields with concentrations of 5 µg/ml inhibiting production of orf-11 and MN viruses and delaying and reducing yields of VV and NZ-2 viruses. Parapoxvirus production was inhibited by 20 µg/ml cidofovir whereas VV production at this cidofovir concentration was detectable although delayed and reduced.

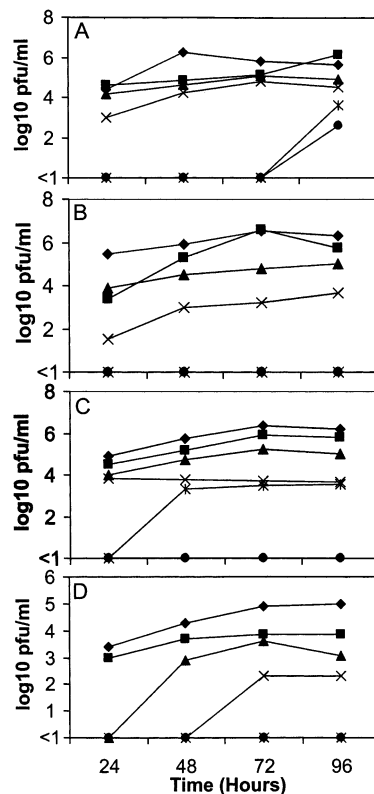


Fig. 1. Yields of vaccinia virus (VV) (A), Orf-11 virus (B), NZ-2 virus (C) and MN virus (D) over time in the presence of 0 (–◆–), 0.2 (–■–), 0.5 (–▲–), 2 (–X–), 5 (–△–) or 20 (–●–) µg/ml of cidofovir.

Results with VV were comparable to an earlier report in which the IC₅₀ was 4 µg/ml in primary rabbit kidney cells (De Clercq et al., 1987). Cidofovir has also been shown to be effective in the treatment of infections caused by the orthopoxviruses VV and cowpox in mice (Neyts and De Clercq, 1993; Bray et al., 2000) and the molluscipoxvirus, molluscum contagiosum in humans (Meadows et al., 1997). The marked in vitro sensitivity of parapoxviruses to cidofovir has not previously been recorded. Parapoxviruses have the highest DNA G-C content (64%) of the known poxviruses (cf. Molluscipoxviruses (60%) and orthopoxviruses (36%)), which could render them more sensitive. Indeed the 64% G-C content is comparable to some herpesviruses (57–69%). Cidofovir is already licensed for clinical use against human cytomegalovirus retinitis in AIDS patients.

During clinical treatment cidofovir confers a long-lasting antiviral response either by injection or topical administration (De Clercq, 1998). This could make it useful for human patients with prolonged or complicated orf and would also make its use in animals more practical. As well as domesticated species, cidofovir may also have a role in the treatment of seals in sanctuaries and an endangered species such as the red squirrel in Britain in which parapoxvirus infection may be a significant factor in its decline (Sainsbury et al., 1997). The encouraging results of these in vitro findings justifies the evaluation of cidofovir as a candidate drug for the treatment of parapoxvirus infections of humans and animals. In fact, cidofovir has been used recently, with striking success, in the case of 'giant orf' of an immunosuppressed patient, where topical cidofovir application resulted in complete regression of the lesion that otherwise would have led to the amputation of the affected finger (Geerinck et al., 2000).

Acknowledgements

We thank members of the Scottish Diagnostic Virology Group for prompting this work, Dr Mathias Buttner for providing MN virus, and Dr.

Simon Wheeler who kindly supplied the cidofovir as Vistide™ (Pharmacia and Upjohn). Funding was provided by Scottish Executive Rural Affairs Department.

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