Short communication

In vitro susceptibility of sea lion poxvirus to cidofovir


A R T I C L E   I N F O

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A B S T R A C T

Parapoxviruses of seals and sea lions are commonly encountered pathogens with zoonotic potential. The antiviral activity of the antiviral compounds isatin-beta-thiosemicarbazone, rifampicin, acyclovir, cidofovir and phosphonoacetic acid against a parapoxvirus (SLPV-1) isolated from a Californian sea lion (Zalophus californianus) was evaluated. Cidofovir was able to reduce virus-induced cytopathic effect of SLPV-1 in confluent monolayers when used in concentrations greater than 2 μg/ml. A decreasing virus yield was observed in the presence of increasing concentrations of cidofovir, which confirmed the ability of cidofovir to inhibit SLPV-1 replication. The in vitro efficacy of cidofovir against SLPV-1 indicates the therapeutic potential of cidofovir for the treatment of infections of humans and pinnipeds with parapoxviruses of seals and sea lions. This study confirms the previously proposed therapeutic potential of cidofovir for the treatment of parapoxvirus infections.

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The poxviruses of pinnipeds have been tentatively classified as parapoxviruses (Osterhaus et al., 1994; Nettleton et al., 1995; Becher et al., 2002; Nollens et al., 2006a). Poxvirus infections are a common complication in the treatment of stranded pinnipeds in marine mammal rehabilitation centers. The pinniped parapoxviruses are considered as zoonotic agents, as several seal handlers have acquired “sealpox” lesions (Hicks and Worthy, 1987; Clark et al., 2005). Other parapoxviruses (orf virus, bovine papular stomatitis virus and pseudocowpoxvirus) are known to infect man as well (Mercer et al., 1997). Infections of humans with the different parapoxviruses, including those of pinnipeds, are clinically indistinguishable. Lesions typically develop on the hands and fingers and usually resolve spontaneously within 6–8 weeks (Shelley and Shelley, 1983; Hicks and Worthy, 1987; Clark et al., 2005). Complications can occur and these include prolonged resolution time, ulceration, secondary bacterial infection, lymphangitis, lymphadenitis and bullous pemphigoid (Yirrell et al., 1991; Murphy and Ralfs, 1996; Reid, 1998).

Orf virus, bovine papular stomatitis virus and pseudocowpoxvirus infections of humans have been successfully treated via cryotherapy (Degraeve et al., 1999), excision of the lesions (Shelley and Shelley, 1983), amputation of the affected finger (Reid, 1998) and cidofovir (Geerinck et al., 1998). The in vitro antiviral activity of cidofovir (S)-1-[(3-hydroxy-2-phosphonylmethoxy)propyl]cytosine against orf virus has been reported (Nettleton et al., 2000). Infections of humans with these parapoxviruses have been effectively treated using local, intranasal or intravenous administration of this drug (De Clercq, 2002a; McCabe et al., 2003).

Other compounds that are known to interfere with the effective replication of a number of DNA viruses include isatin-beta-thiosemicarbazone (IBT), rifampicin, phosphonoacetic acid (PAA) (foscarnet) and the nucleoside analogue acyclovir (9-(2-hydroxyethoxymethyl)guanine). IBT inhibits viral transcription and in vitro growth of vaccinia virus and cowpox virus...
Poxvirus (SLPV-1; Nollens et al., 2006b) isolated from a Californian antiviral activity of these compounds against the parapoxviruses of pinnipeds. The Sea lion (Zalophus californianus) as a model virus for testing the in vitro efficacy of IBT, rifampicin, acyclovir, cidofovir and PAA against the parapoxviruses of pinnipeds.

SLPV-1 was propagated in early passage California sea lion kidney (CZC-K) and a purified SLPV-1 stock was prepared on a Na-diatrizoate density gradient as previously described (Nollens et al., 2006b). This virus stock was used to evaluate sensitivity to compounds at the various concentrations after which the presence or absence of CPE was recorded. IBT, rifampicin or acyclovir did not inhibit CPE formation. One and two infected wells incubated in the presence of 5 and 20 μg/ml cidofovir, respectively, were CPE-negative. Additionally, no infected well incubated in the presence of 200 μg/ml PAA was CPE-negative. The antiviral inhibitory activity of cidofovir and PAA was confirmed via a duplicate experiment using early passage California sea lion skin (CZC-S) cells following the exact same protocol. CPE reduction was confirmed using cidofovir at concentrations ≥ 2 μg/ml and when using PAA at concentrations > 200 μg/ml. Antiviral activity was expressed as the minimum antiviral concentration (IC50), defined as the compound concentration needed to prevent CPE in two or more wells. At any given concentration, PAA was unable to prevent CPE in more than one well. The IC50 for CPE of cidofovir was estimated between 5 and 20 μg/ml and between 2 and 5 μg/ml when using Californian sea lion kidney and skin cell cultures, respectively.

To evaluate the effect of cidofovir on SLPV-1 yield, CZC-S monolayers of one drug-free control (0 μg/ml; Fig. 1) and one well incubated at each drug concentration were harvested and the IC50 virus titer was determined. After 5 days of incubation, the calculated virus yield of cells infected in the absence of cidofovir (0 μg/ml) was 10^6.8 CCID50/ml. Virus yield decreased with increasing concentrations of cidofovir. The SLPV-1 yield of infected cells incubated in the presence of 0.2, 0.5 μg/ml of cidofovir was 10^6.8, 10^6.0 CCID50/ml, respectively. The SLPV-1 yield of infected cells incubated in the presence of 2, 5 and 20 μg/ml of cidofovir was reduced to 10^6, 10^5.1 and 10^4.1 CCID50/ml, respectively; or 13.4, 2.0 and 0.1% of the virus yield of the drug-free control.

A cytotoxic effect of the antiviral agent to CZC-S monolayers could be falsely interpreted as virus-induced CPE. The cytotoxic effect of cidofovir was therefore evaluated on both confluent and exponentially growing CZC-S cells. Triclate confluent CZC-S monolayers were incubated for 5 days in the presence of culture media containing cidofovir at 0, 0.2, 0.5, 2, 5 and 20 μg/ml. After 5 days of incubation at 37.0 °C, the presence or absence of cytotoxicity, defined as apparent CPE, was recorded. No cytotoxicity was observed in the drug-free CZC-S control wells, but one of three monolayers incubated in the presence of cidofovir at a concentration of 20 μg/ml did show cytotoxicity.

**Fig. 1.** Sea lion parapoxvirus (SLPV-1) yield (±S.E.) of infected CZC-S cells incubated in the presence of 0, 0.2, 2, 5 or 20 μg/ml of cidofovir for 5 days. A decreasing virus yield was observed in the presence of increasing concentrations of cidofovir.

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**References:**

2. Rifampicin interferes with the function of a structural protein of vaccinia virus and consequently inhibits the maturation of virions (Sajner et al., 2005). PAA is a pyrophosphate analogue with activity against various DNA viruses via the inhibition of viral DNA polymerases (Crumpacker, 1992). Acyclovir and cidofovir are, respectively, guanine and cytosine nucleoside and nucleotide analogues that are FDA-approved for the treatment of human herpesviral infections. Cidofovir is converted exclusively via cellular enzymes to its active diphosphate form, whereas acyclovir requires both viral and cellular kinases to be phosphorylated into its active form (Elion, 1983; Morfin and Thouvenot, 2003). The active forms of acyclovir and cidofovir stop viral DNA replication via incorporation into and termination of the DNA chain, and via inactivation of the DNA polymerase (Elion, 1983; Magee et al., 2005). The antiviral activity of these compounds against the parapoxviruses of pinnipeds has not yet been determined. In this study we used a poxvirus (SLPV-1; Nollens et al., 2006b) isolated from a Californian sea lion (Zalophus californianus) as a model virus for testing the in vitro efficacy of IBT, rifampicin, acyclovir, cidofovir and PAA against the parapoxviruses of pinnipeds.
and higher. Zoonotic orf virus infection has been successfully ofovir exhibited anti-SLPV-1 activity at concentrations of 2 µg/ml. No significant cytotoxic effect of cidofovir on cell growth was detected (p > 0.05, Student's t-test).

Our findings showed an unequivocal antiviral effect of cidofovir (55, 1–13). We therefore recommend cidofovir as a candidate therapeutic for the treatment of zoonotic sealpox infections as well. Cidofovir is presently of limited use for the treatment of parapoxvirus infections of wild seals and sea lions due to the need for handling and other practical constraints. Clinical trials are currently underway to test the safety and efficacy of CMX001, an ether-lipid analogue of cidofovir with excellent oral bioavailability (Buller et al., 2004; Quenelle et al., 2004; Parker et al., 2008). CMX001 may be an excellent candidate for the treatment of these parapoxvirus-infected pinnipeds.

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