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Cidofovir is effective against caprine herpesvirus 1 infection in goats

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Abstract

Caprine herpesvirus 1 (CpHV-1) is a virus able to cause genital infection leading to vulvovaginitis or balanoposthitis in adult goats. CpHV-1 shares several biological similarities with herpes simplex type 2 (HSV-2) infection in man, such as genital tropism, type and site of typical lesions and it might provide an animal model for studies on antiviral drugs for HSV-2 infection in man. In this view the efficacy of cidofovir (CDV) drug was tested in six goats intravaginally infected with BA.1 strain of CpHV-1. Three goats received an intravaginal application of 3 ml of a 1% CDV preparation at 4 h post infection and then every 12 h for five consecutive days. Three goats were kept as untreated controls. The goats were daily examined for clinical evidence of the infection and viral shedding. CDV was able to protect against disease progression and inhibited the onset of the local lesions due to the CpHV-1 replication. Treated animals shed virus for a shorter period (3 days less) and at lower titres than the control animals. CpHV-1 infection in goats may represent an excellent animal model for the study of novel strategies for the treatment of primary genital HSV-2 infection in man.

Keywords: Cidofovir; Antiviral drug; Caprine herpesvirus 1; Goat; Animal model; Herpes simplex virus 2

1. Introduction

Caprine herpesvirus 1 (CpHV-1) is an alphaherpesvirus of goats widespread in the goat population throughout the world with a seroprevalence of 30–40% in the Mediterranean countries. In kids it causes a systemic disease characterised by high morbidity and mortality rates (Saito et al., 1974; Mettler et al., 1979; Roperto et al., 2000) with ulcerative and necrotic lesions distributed throughout the enteric tract (Van der Lugt and Randles, 1993). In adult goats, the infection leads to vulvovaginitis or balanoposthitis (Horner et al., 1982; Grewal and Wells, 1986; Tarigan et al., 1987). Abortions associated with CpHV-1 occur during the second half of pregnancy and can be experimentally reproduced after intranasal or intravenous inoculation of pregnant goats (Waldvogel et al., 1981; Williams et al., 1997; Tempesta et al., 2004a). Natural reactivation of CpHV-1 infection is generally observed during the mating season only in animals with low antibody titres (Tempesta et al., 1998a). The experimental reactivation is difficult and is obtained using high dosage of dexamethasone and in animals with low antibody titres (Plebani et al., 1983; Buonavoglia et al., 1996). Viruses are isolated from nasal, genital, rectal and ocular swabs, suggesting several potential latency sites, such as sacral ganglia where latent CpHV-1 has been detected by PCR (Tempesta et al., 1999a,b, 2002). The site supporting CpHV-1 re-excretion may depend on the route of primary infection. After reactivation of a virus previously inoculated intranasally, the virus is shed from the nose and vagina. In contrast, after a genital primary infection, reactivated CpHV-1 is only re-excreted from the vagina (Tempesta et al., 2002).

CpHV-1 shares several biological similarities with HSV-2 infection in man, such as genital tropism, type and site of typical lesions (erythematosous confluent vesicles evolving in superficial ulcers) and latency in sacral ganglia (Tempesta et al., 1999b). In this view CpHV-1 infection may represent an animal model for studies on antiviral drugs for HSV-2 infection in man.

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Cidofovir (CDV) is an acyclic nucleoside phosphonate having potent broad spectrum anti-DNA virus activity. CDV have been approved for the treatment of CMV retinitis in AIDS patients and licensed for clinical use under the trade name of Vistide from 1996 (De Clercq, 2004). It has also shown to be effective in the treatment of genital warts, laryngeal and cutaneous papillomatous lesions, molluscum contagiosum lesions, orf lesions, adenovirus infections and progressive multifocal leukoencephalopathy (PML) (De Clercq, 2004, 2005). The topical use of CDV for the treatment of viral skin diseases has been demonstrated in animals and in a limited number of humans (Zabawsky, 2000). The aim of this preliminary study is to investigate the efficacy of a topical (vaginal) administration of CDV in goat with CpHV-1 infection.

2. Materials and methods

2.1. Animals

Six 6 years old Maltesian cross breed goats seronegative to CpHV-1, belonging to a CpHV-1 free flock were used in this experiment. Three goats were infected with CpHV-1 and treated with CDV; the other three animals were kept as control and only infected with CpHV-1.

2.2. Virus

BA.1 strain of CpHV-1 (Buonavoglia et al., 1996) was cultivated on Madin Darby bovine kidney cells (MDBK) grown in Dulbecco-Minimal Essential Medium (D-MEM) supplemented with 10% foetal calf serum. The virus titres was $10^{6.50}$ Tissue Culture Infectious Doses ($TCID_{50}$)/50 μl.

2.3. Cidofovir

CDV was kindly provided by Dr. W. Lee, Gilead Sciences, Foster City, CA. CDV cream formulated in 1% in Beeler base (cetyl alcohol 15 g, white wax 1 g, propyleneglycol 10 g, sodium lauryl sulphate 2 g and water 72 g) was used in this study (Stragier et al., 2002). In a previous experiment, in two non-infected goats, a 1% CDV cream administered intravaginally once daily (3 ml per administration) for five consecutive days, did not induce local or systemic alterations (data not shown).

2.4. Experimental design

The goats were sedated with xilazine 0.05 mg/kg (Rompum, Bayer S.p.A., Milan, Italy), and, after 15 min they were intravaginally infected with 4 ml of BA.1 strain of CpHV-1 (titre: $10^{6.50}TCID_{50}$/50 μl) as previously reported (Tempesta et al., 2002). Three goats (T1–T3) received an intravaginal application of 3 ml of a 1% CDV preparation at 4 h post infection and then every 12 h for five consecutive days. Three goats (C1–C3) were kept as control and similarly treated intravaginally with cream without CDV. All the animals were kept under observation for 20 days. The goats were daily examined for clinical evidence of the infection; temperature was taken and general and local signs were scored. Clinical signs, hyperemia, oedema, lesions and pain were graded as 0 (absent); 1 (mild); 2 (moderate), and 3 (severe). Temperature elevations above normal (38.2–38.6 °C) were graded: >0.5–1 °C = 1; 1.1–1.5 °C = 2; >1.5 °C = 3. The total daily clinical score for each animal was recorded and used to draw the final graphic. Vaginal swabs were collected from the 1st day before the infection and then before each administration of CDV and, after CDV treatment (from 6th day), once daily between day 6 and 20 post infection. Blood samples were taken at day 0, and 20 days later to evaluate antibody titres to CpHV-1 by means of seroneutralization test (SN), as previous reported (Buonavoglia et al., 1996).

2.5. Polymerase chain reaction (PCR)

Viral DNA was extracted from swabs using the commercial kit QIAamp Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the instructions of the Company. PCR was carried out following the protocol described by Tempesta et al. (1998b). A pair of primers corresponding to the sequences 632–653 and 1046–1027 of the gene coding for the glycoprotein C (gC) of BA.1 strain of CpHV-1 was chosen (Tempesta et al., 2004b). PCR was carried out in a total volume of 25 μl containing 5 μl of DNA sample, 2.5 μl of PCR buffer 10×, 1.5 mM $MgCl_2$, 1.25 mM of each oligonucleotide triphosphate, 200 μM of each primer, 2.5 U of Ampli Taq Gold polymerase (Perkin-Elmer, Norwalk, USA), 2.5 μl of glycerol and sterile water up to 25 μl. The thermal profile consisted of a 10 min at 94 °C, 40 cycles at 94 °C for 1 min (denaturation), 70 °C for 1 min (annealing) and 72 °C for 1 min (polymerisation) followed by a final extension at 72 °C for 10 min. Ten microliters of the PCR products were analysed by electrophoresis in 1.5% agarose gel and visualised by UV light after ethidium bromide staining.

2.6. Virus isolation and titration

Vaginal swabs (to which antibiotics were added at a final concentration of 5000 UI/ml penicillin, 2500 μg/ml streptomycin and 10 μl/ml amphoterycin B) were serial 10-fold diluted and inoculated in quadruplicate onto MDBK cells in 96-wells microtitres plates. The plates were read after 3 days of incubation and viral titres were calculated by Reed-Muench method.

2.7. Data analysis

Data concerning clinical scores, titres of virus shedding from vagina of the goats were analysed by GraphPad prism Version 3.00 software (GraphPad Software, San Diego, CA, USA) by calculating the area under the curve (AUC). The groups of animals have been statistically compared by using Student's *t*-test.

3. Results

3.1. Clinical examination

During the observation period the three CDV-treated goats did not show any general clinical signs. They only showed an

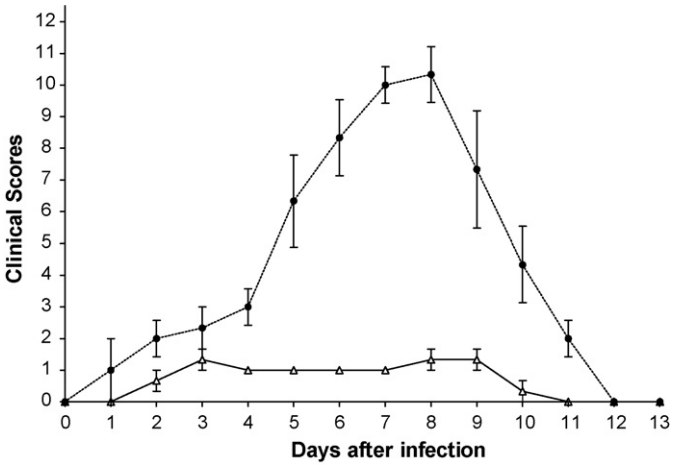

Fig. 1. Effect of CDV on disease progression in goats following CpHV-1 genital infection. Arithmetic means of clinical score in untreated (●) and treated (△) goats.

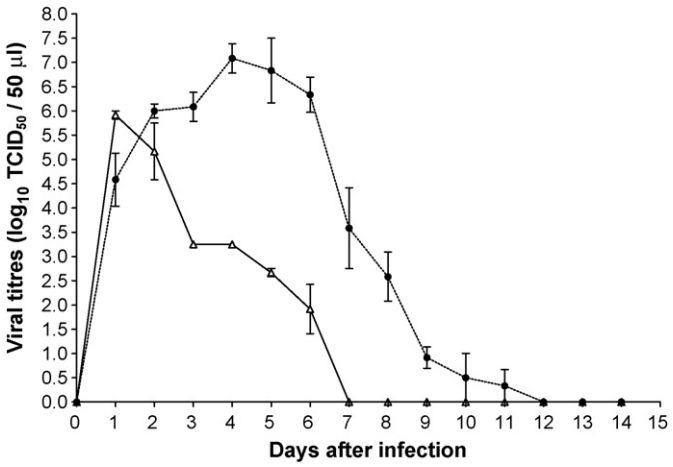

Fig. 2. CpHV-1 titres in vaginal samples of CDV untreated (●) and treated (△) goats.

increase of temperature for one day (2nd or 3rd day) and a slight vaginal hyperaemia lasting 6–8 days. The goats did not suffer from topical lesions typical of CpHV-1 infection. Erythema, oedema and pain at swabbing were not observed. The infected not-treated goats developed, as expected, high temperature for 8–10 days, and, at the vaginal level, severe hyperaemia lasting 6–9 days with the extreme from days 2 to 10, severe oedema and extensive ulcerative lesions lasting up to the 11th d.p.i., with local pain at the swabbing (Fig. 1). The clinical score AUC values in cidofovir-treated goats were 11.0, 8.0 and 8.0 whereas in control goats were 57.0, 50.0 and 64.0 showing a very high significance ($P = 0.0003$).

3.2. Virus isolation

The CDV-treated goats shed virus from the 1st to the 6th day post infection (d.p.i.) with the peak of viral excretion ($10^{6.00}$TCID$_{50}$/50 μl) at d.p.i. 1. The control goats shed virus from the 1st to the 11th d.p.i. (C1 up to the 9th d.p.i.) with the peak of viral excretion ($10^{7.50}$TCID$_{50}$/50 μl) at days 3–5 d.p.i. (Fig. 2). In treated animals the AUC values were 22.00, 20.75 and 23.75 TCID$_{50}$/50 μl/day, whereas in the control untreated goats, were 47.50, 40.25 and 46.75 TCID$_{50}$/50 μl/day showing a high significance ($P = 0.0008$). Viral DNA was detected by PCR up to the 6th d.p.i. in treated goats and to the 12th in one of the controls.

3.3. Antibody values

All goats were seronegative at time 0. Twenty days after challenge, neutralizing antibody titres in the CDV treated goats ranged from 1:8 to 1:32. The not-treated goats had a SN titres ranging from 1:16 to 1:32.

4. Discussion

CDV has been already experimentally used by systemic administration for herpesvirus infections in animals. It has been shown to be effective in vitro against equine herpesvirus 1 (EHV-1) (Gibson et al., 1992, 1993), bovine herpesvirus 1 (BHV-1) (Gilliam and Field, 1993) and feline herpesvirus 1 (Maggs and Clarke, 2004). Regarding the efficacy of CDV in large animals, Gibson et al. (1992) reported that a single subcutaneous administration (20 mg/kg) of CDV in horses on the day of intranasal EHV-1 infection markedly reduced clinical signs and virus shedding. Gilliam and Field (1993) observed that a single dose (20 mg/kg) of CDV administered to calves either the day before or the day following BHV-1 inoculation markedly reduced clinical signs and virus replication.

In our study topical CDV treatment by intravaginally administration every 12 h for five consecutive days to CpHV-1 infected goats was able to protect against disease progression and inhibited the onset of the local lesions due to the virus replication. Nevertheless, according to previous studies, it cannot be ruled out that CDV treatment has not prevented the establishment of virus latency. Gilliam and Field (1993) reported that a single dose of CDV, administered the day before a course of dexamethasone did not prevent virus shedding from calves, which 6 weeks before, were infected with BHV-1 and treated with CDV.

In our experiment, the clinical score AUC revealed significant differences between the treated and control goats ($P = 0.0003$). Regarding viral shedding, treated animals shed virus for a shorter period (3–5 days less) and at lower titres than the control animals: the CDV-treated animals AUC values were lower ($P = 0.0008$) than in control goats. This reduction, in terms of duration and total amount of viral shedding, could have repercussion on (i) reproductive performances of animals and on (ii) circulation of virus among susceptible individuals. CDV has clearly demonstrated a strong antiviral activity against CpHV-1 and it was able to provide a complete clinical protection in the treated goats. Treatment was well tolerated, indicating that CDV could be used for the topical treatment of genital CpHV-1 infections in goats. Nevertheless the cost/benefit ratio makes antiviral therapy not practicable in farm animals. Moreover CpHV-1 infection in goats may represent an excellent animal model to improve new approaches for the treatment of genital HSV-2 infection in man.

Indeed, vaginal infection in goat by CpHV-1 is (i) methodically and easily reproducible, being characterized by evident lesions; (ii) topical application of drugs is easy to perform; (iii) clinical scoring of the infection is very simple to evaluate; (iiii) the grading of the lesions is easy to be pointed out. On the whole, the results of the present study are not applicable, because of the costs, to the treatment of CpHV-1-induced vaginitis in goats. However, these findings raise interesting perspectives on new possibilities of treatment with CDV of genital lesions associated with HSV-2 infection in humans.

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