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In vitro susceptibility of six isolates of equine herpesvirus 1 to acyclovir, ganciclovir, cidofovir, adefovir, PMEDAP and foscarnet

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Abstract

Equine herpesvirus 1 (EHV-1) is an important equine pathogen that causes respiratory disease, abortion, neonatal death and paralysis. Although vaccines are available, they are not fully protective and outbreaks of disease may occur in vaccinated herds. Therefore, there is an urgent need for effective antiviral treatment. For three abortigenic (94P247, 97P70 and 99P96) and three neuropathogenic isolates (97P82, 99P136 and 03P37), the effect of acyclovir, ganciclovir, cidofovir, adefovir, 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) and foscarnet on plaque number was studied. Additionally, for isolate 97P70, the effect on plaque size was investigated. Ganciclovir was most potent in reducing plaque number, followed by PMEDAP and acyclovir. Adefovir and cidofovir were less effective and foscarnet was the least effective compound. There were no differences detected for acyclovir, ganciclovir, adefovir and PMEDAP between the abortigenic and neuropathogenic isolates. One abortigenic isolate (99P96) was more susceptible to cidofovir and two neuropathogenic isolates (99P136 and 03P37) were less susceptible to foscarnet. For isolate 97P70, all compounds resulted in a significant reduction of plaque size. The most remarkable effect was observed for cidofovir. It was 40-fold more effective in reducing plaque size than in reducing plaque number. In conclusion, ganciclovir was the most potent compound and therefore, may be a valuable candidate for the treatment of EHV-1 infections in horses. The antiviral effect of foscarnet on plaque number was highly dependent on the viral isolate tested. Therefore, it is no valuable antiviral for the treatment of herpesvirus-infections. Cidofovir, although less effective in reducing plaque number, had a strong effect on plaque size.

Keywords: EHV-1; Antivirals; *In vitro*; Isolates

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1. Introduction

Equine herpesvirus 1 (EHV-1), a member of the Alphaherpesvirinae, is a major pathogen of horses. The virus is endemic worldwide and most horses become infected during their first year of life (Ostlund, 1993). After exposure, EHV-1 replicates in the upper respiratory tract. This can be associated with respiratory disorders, characterised by fever, anorexia, nasal discharge of varying severity and ocular discharge (Patel and Heldens, 2005), or the infection can be silent (Foote et al., 2006). Replication is followed by a leukocyte-associated viremia which enables EHV-1 to reach internal organs. There, its replication can result in abortion, neonatal death or nervous system disorders (Allen and Bryans, 1986; Bryans and Allen, 1989). Abortion can involve only one or two mares in a herd but also abortion storms can occur on a premise, associated with great economic losses (Allen and Bryans, 1986). Neurological disorders have been reported with increasing frequency (McMartan et al., 1995; Friday et al., 2000; van Maanen et al., 2001; Stierstorfer et al., 2002; van der Meulen et al., 2003a) and during an outbreak many cases may occur with devastating effects.

Although vaccines against EHV-1 are available, they are not fully protective (Kydd et al., 2006; Goodman et al., 2006; van der Meulen et al., 2006a) and outbreaks of disease may still occur (Buchner and Mostl, 1998; Friday et al., 2000; van der Meulen et al., 2000; Kohn et al., 2003; Goehring et al., 2006). Therefore, there is a need for effective antiviral chemotherapy. Antivirals need to meet several requirements. They have to reduce viral replication and spread in affected horses, and prevent viral replication in in-contact animals. The drug should be effective against isolates of EHV-1 associated with outbreaks of abortion as well as those associated with outbreaks of neurological disorders. Recently, it was postulated that a variation of a single amino acid of the DNA polymerase is strongly associated with neurological versus non-neurological disease outbreaks (Nugent et al., 2006). This may have an implication on the susceptibility of various isolates to anti-herpetic compounds as many of these compounds act on the DNA polymerase (De Clercq, 2004). Finally, the drug should be safe and devoid of adverse effects.

Some antivirals have already been tested *in vitro* for their efficacy to inhibit EHV-1 replication by means of a plaque reduction assay, *i.e.* ganciclovir (Smith et al., 1983; Rollinson and White, 1983; Rollinson, 1987), three 2′-fluoropyrimidine nucleosides (Rollinson, 1987), (*S*)-9-[3-hydroxy-2-phosphonylmethoxypropyl]adenine or HPMPA (Field and Awan, 1990) and the HPMPA analogue cidofovir (Gibson et al., 1992). These compounds proved more effective than 9-[4-hydroxy-3-hydroxymethylbut-1-yl]guanine (Boyd et al., 1987) and penciclovir (de la Fuente et al., 1992). For acyclovir, contradictory data have been reported. Rollinson and White (1983) reported a 6-fold lower EC_{50} than Boyd et al. (1987). Further, based on comparison of EC_{50}-values reported in literature, adefovir would be the least active compound in this series (Field and Awan, 1990). However, data of these different studies are difficult to compare as different isolates, cell lines and assays have been employed.

The aim of the present study was to compare the efficacy of acyclovir, ganciclovir, cidofovir, adefovir, PMEDAP and foscarnet against EHV-1 *in vitro*. Four of these products have already been tested *in vitro* while foscarnet and PMEDAP are new in this study. Three abortigenic and three neuropathogenic isolates of EHV-1 were included and one cell line, EEL cells, was used. Additionally, the efficacy of the antivirals to reduce the size of EHV-1 induced plaques was investigated. Reduction in plaque size may be a potential parameter for the ability of an antiviral to inhibit cell to cell spread and, consequently to restrict the size of macroscopic lesions *in vivo*.

2. Materials and methods

2.1. Cells

All studies were conducted using equine embryonic lung (EEL) cells. EEL cells were cultured in growth medium (minimum essential medium (MEM) supplemented with 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.1 mg/ml kanamycin, 0.3 mg/ml glutamine and 5% foetal calf serum). Cells were passaged once a week.

2.2. Viruses

Six Belgian EHV-1 isolates were tested. The isolates 94P247, 97P70 and 99P96 were isolated