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Review

Antiviral agents against equid alphaherpesviruses: Current status and perspectives

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ABSTRACT

Equid herpesvirus infections cause respiratory, neurological and reproductive syndromes. Despite preventive and control measures and the availability of vaccines and immunostimulants, herpesvirus infections still constitute a major threat to equine health and for the equine industry worldwide. Antiviral drugs, particularly nucleoside analogues and foscarnet, are successfully used for the treatment of human alphaherpesvirus infections. In equine medicine, the use of antiviral medications in alphaherpesvirus infections would decrease the excretion of virus and diminish the risk of contagion and the convalescent time in affected horses, and would also improve the clinical outcome of equine herpesvirus myeloencephalopathy. The combined use of antiviral compounds, along with vaccines, immune modulators, and effective preventive and control measures, might be beneficial in diminishing the negative impact of alphaherpesvirus infections in horses. The purpose of this review is to analyse the available information regarding the use of antiviral agents against alphaherpesviruses, with particular emphasis on equine alphaherpesvirus infections.

Introduction

Herpesviruses (order *Herpesvirales*, family *Herpesviridae*) cause severe disease in a broad range of mammals, including humans. The viruses are widely distributed, and among their biological properties is the ability to establish latent infections in their hosts. During reactivation from latency, herpesviruses can cause recrudescence of disease, and are always re-excreted to the environment (Pellet and Roizman, 2007). Within this family, equid herpesvirus (EHV)-1, EHV-4 and EHV-3 are alphaherpesviruses (subfamily *Alphaherpesvirinae*, genus *Varicellovirus*) that affect horse populations on all continents (Davison et al., 2009; Davison, 2010; Ma et al., 2013).

EHV-1 infection is frequently associated with respiratory disease, abortion, perinatal foal mortality and/or neurological disease (equine herpesvirus myeloencephalopathy [EHM]) (Allen et al., 2004; Lunn et al., 2009; Goodman et al., 2012; Ma et al., 2013; Pusterla and Hussey, 2014). Outbreaks resulting in multiple fetal losses in a very short period of time (abortion storms), with abortion rates as high as 75%, have been recorded (Barrandeguy et al., 2002; Allen et al., 2004; Slater, 2007, 2014; Pusterla and Hussey, 2014). A substantial increase in the incidence of EHM has been documented worldwide during the last decade and it is now considered the most common cause of neurological problems in horses (Lunn et al., 2009; Slater, 2014). The occurrence of EHM is significantly, but not exclusively, associated with viral strains carrying a single nucleotide polymorphism at position 2254 (A/G) of the viral polymerase gene (ORF30) (Nugent et al., 2006; Vissani et al., 2009; Pronost et al., 2010a, 2010b).

EHV-4 infection causes upper respiratory tract disease in foals but is clinically indistinguishable from that caused by EHV-1, and has been very occasionally associated with the development of viral bronchopneumonia and abortions (Allen et al., 2004; Slater, 2007; Ma et al., 2013). EHV-3 is the causative agent of equine coital exanthema (ECE), an acute venereal and highly contagious disease, characterised by the development of superficial papules, vesicles, pustules and ulcers on the external genitalia of both mares and stallions (Allen and Umphenour, 2004; Barrandeguy and Thiry, 2012).

Although vaccines against EHV-1 and EHV-4 are available, they are mostly effective at generating serum virus-neutralising antibodies (Goodman et al., 2012; Kydd et al., 2012), and are therefore not fully protective. Outbreaks of disease may occur even in vaccinated herds (Allen et al., 2004; Kydd et al., 2012). Furthermore, there is no evidence that current vaccines can prevent naturally occurring cases of EHM (Lunn et al., 2009; Pusterla and Hussey, 2014; Slater, 2014), and none is completely effective at eliminating nasal virus shedding or cell-associated viraemia (Patel and Heldens, 2005; Osterrieder, 2007; Goodman et al., 2012; Kydd et al., 2012). There are no currently licensed vaccines against ECE, and preventive and

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control measures basically rely on segregation of affected mares and stallions from reproductive activities. Such measures greatly contribute, but do not eliminate, the risk of contagion, since EHV-3 is subclinically re-excreted from latently infected animals upon viral reactivation (Barrandeguy et al., 2010, 2012; Barrandeguy and Thiry, 2012).

Concurrently, three immunostimulant products, inactivated *Parapoxvirus ovis*, inactivated *Propinebacterium acnes* and a purified cell-wall extract, are available for use in horses either with or at risk of developing infectious diseases (Cormack et al., 1991; Paillot, 2013). Despite the existence of vaccines and immunostimulant products and the application of sound preventive measures, outbreaks of EHM, abortions (Vissani et al., 2009; Pronost et al., 2010b; Smith et al., 2010; Gryspeerdt et al., 2011; Traub-Dargatz et al., 2013; Pusterla and Hussey, 2014) and ECE (M. Barrandeguy, personal communication) are still frequently reported worldwide.

In humans, alphaherpesvirus infections are successfully treated with antiviral drugs (De Clercq, 2008; Razonable, 2011) that reduce the convalescent period and the rate of transmission to naïve in-contact individuals by decreasing the availability of infectious viral particles capable of infecting new cells and limiting viral shedding to the environment (Flint et al., 2004; Coen and Richman, 2007). In equine medicine, the use of antiviral compounds may be beneficial in diminishing the negative impact of alphaherpesvirus infections (Garre, 2008; Maxwell et al., 2009, 2011; Glorieux et al., 2012; Carmichael et al., 2013).

The purpose of this review is to analyse the available information regarding the use of antiviral agents against alphaherpesviruses, with particular emphasis on equine alphaherpesvirus infections.

Antiviral compounds for alphaherpesviruses: Mechanisms of action

Herpesviruses are complex viruses that encode between 70 and 200 genes in their genome. The genes involved in viral replication are classified as 'essential' or 'non-essential' depending on whether they are necessary or not for viral replication in cultured cells (Roizman et al., 2007). Essential viral proteins required for DNA replication are the DNA polymerase (an origin-binding protein), the single-stranded DNA binding protein, and the DNA helicase–primase complex (Roizman et al., 2007). Non-essential viral proteins include the enzymes thymidine kinase (TK) and ribonucleotide reductase. Both essential and non-essential viral enzymes differ sufficiently from their cellular counterparts, and this has allowed the development of compounds that can specifically inhibit the viral enzymes involved in replication. The herpes simplex virus DNA polymerase interacts with a broader range of deoxynucleoside triphosphates than cellular polymerases (Roizman et al., 2007). On the other hand, viral TK differs from the corresponding cellular TK in biochemical, immunological, and substrate specificities (Karlstrom et al., 1986; Evans et al., 1998). Viral TK phosphorylates pyrimidines and even purine nucleosides, carries out multiple phosphorylations on thymidine, and uses nucleotide triphosphates other than ATP as source of phosphate. Moreover, the mutability of the enzyme appears to be greater than that of the cellular TK (Evans et al., 1998; Cohen et al., 2007).

Nowadays, all of the antiviral agents available for the treatment of alphaherpesvirus infections, except for foscarnet, are nucleoside analogues, which can be classified into three groups, namely, acyclic guanosine analogues, acyclic thymidine analogues and acyclic nucleoside phosphonates, which mimic deoxynucleoside deoxyguanosine, thymidine triphosphate and deoxynucleotide deoxycytidine monophosphate, respectively. Nucleoside analogues need to be phosphorylated to the triphosphate form, before they can interact with DNA polymerase (De Clercq, 2002; Coen and Richman, 2007).

For acyclic guanosine and thymidine analogues, the first (and for brivudin also the second) phosphorylation step is ensured by virus-encoded TK, which explains the specific anti-herpes action of these compounds. Subsequent phosphorylations are achieved by host cellular kinases (De Clercq, 2002). In contrast, acyclic nucleoside phosphonates require only two phosphorylation steps to be converted into their active metabolites, as their structure contains a phosphonate moiety, which is equivalent to a phosphate. Thus, these compounds are active against DNA viruses that do not encode a specific viral TK, or that have become resistant to nucleoside analogues through TK deficiency (De Clercq, 2002; Coen and Richman, 2007).

In their active form (triphosphate, for acyclic guanosine and thymidine analogues, and diphosphate for acyclic nucleoside phosphonates), nucleoside analogues interact with viral polymerases by competing with the natural dNTP substrate (competitive inhibition) or by being incorporated as an alternative substrate. Either or both of these mechanisms (competitive inhibition and/or incorporation) are essential for antiviral activity (De Clercq, 2002; Coen and Richman, 2007). As acyclic guanosine analogues (acyclovir, penciclovir and ganciclovir) have a limited oral bioavailability, they have been replaced by their prodrugs (valaciclovir, famciclovir and valganciclovir, respectively) in oral treatments. Prodrugs undergo biological or chemical transformation in vivo resulting in the release of the biologically active agent that elicits the desired pharmacological effect (Sofia, 2013).

In contrast to nucleoside analogues, foscarnet is an analogue of pyrophosphate, which is a product of polymerisation of nucleic acids, and therefore does not require activation by either cell or viral enzymes but rather inhibits DNA polymerase directly (Coen and Richman, 2007). This inhibitory mechanism responsible for the antiviral effects is achieved by binding to the site occupied by pyrophosphate, preventing normal pyrophosphate release. In turn, the DNA polymerase cannot complete the catalytic cycle successfully (Coen and Richman, 2007).

Antiviral compounds for human alphaherpesviruses: Past and present

Human herpes simplex virus (HSV)-1, the prototype member of the subfamily *Alphaherpesvirinae*, was the first virus successfully treated using antiviral compounds. The nucleoside analogues idoxuridine (Prusoff, 1959) and trifluridine (Kaufman and Heidelberger, 1964) were the first drugs synthesised for the treatment of herpetic keratitis. Acyclovir, developed in the late 1970s (Elion et al., 1977), showed very high specificity for HSV and varicella-zoster virus (VZV), being remarkably safer for intravenous (IV), oral or topical administration than previous nucleoside analogues (Flint et al., 2004; Coen and Richman, 2007).

Further developments in herpesvirus chemotherapy include the discovery of other nucleoside analogues, such as brivudin, ganciclovir, and penciclovir, as well as other analogues targeting the viral DNA polymerase, such as the nucleoside phosphonate analogues, including cidofovir, and the pyrophosphate analogues, including foscarnet. Nucleoside oral prodrugs, such as valaciclovir (prodrug of acyclovir), famciclovir (prodrug of penciclovir) and valganciclovir (prodrug of ganciclovir), which show increased oral bioavailability, have also been developed (De Clercq, 2004a; Flint et al., 2004). Improvements are ongoing with new congeners of nucleoside analogues, as well as on non-nucleoside herpesvirus DNA polymerase inhibitors, and inhibitors of the HSV helicase/primase complex (Neyts et al., 2001; Chono et al., 2010; Glorieux et al., 2012).

Antiviral drugs currently approved by the United States Food and Drug Administration (FDA) for the treatment of HSV-1 and HSV-2 infections are acyclovir, valaciclovir, penciclovir, famciclovir, idoxuridine, trifluridine and brivudin; the first four are used to treat mucocutaneous disease (De Clercq, 2008; Razonable, 2011). The