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Prophylactic and therapeutic intervention of Punta Toro virus (*Phlebovirus*, Bunyaviridae) infection in hamsters with interferon alfacon-1

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Abstract

Punta Toro virus (PTV) is a member of the Bunyaviridae family, genus *Phlebovirus*, related to the highly pathogenic Rift Valley fever virus (RVFV). It produces a disease in hamsters that models severe Rift Valley fever (RVF) in humans. The recent outbreak of RVF in Kenya stresses the need to identify prophylactic and therapeutic measures for preventing and treating severe forms of disease. To this end, interferon (IFN) alfacon-1 (consensus IFN-α) was evaluated in cell culture against RVFV and PTV, and in the hamster PTV infection model. Survival outcome following treatment initiated pre- and post-virus challenge and the suppression of viral burden and liver disease in infected hamsters was determined. Pre-treatment of cell cultures with IFN alfacon-1 induced marked antiviral activity against both viruses. Intraperitoneal treatment of hamsters initiated 4 h prior to infection with PTV was highly protective and greatly limited liver disease and systemic and liver viral burden. Complete protection from a highly lethal challenge dose was afforded by treatment initiated 36 h following viral inoculation. Although efficacy was much reduced, IFN alfacon-1 therapy was still beneficial when started as late as 3–5 days post-virus exposure. These studies suggest that IFN alfacon-1 may be an effective treatment for early intervention following infection with RVFV.

Keywords: Punta Toro virus; Phlebovirus; Interferon; Interferon alfacon-1; Antiviral; Hamster

1. Introduction

Several viruses of the *Phlebovirus* genus, Bunyaviridae family, are known to cause significant disease in humans. These viruses are maintained in nature in wild or domesticated mammals and are transmitted through feeding of phlebotomous insects. Consequently, phleboviral disease is closely linked to factors that influence host and vector populations. Infection by certain strains of sandfly fever virus (SFFV) has historically been a disease of considerable importance to the military. During World War II, many of the ~19,000 soldiers infected with SFFV required medical attention and hospitalization (Hertig and Sabin, 1964). Although rarely fatal, SFFV infection can result in acute intense fever, severe myalgias, nausea, vomiting, abdominal pain and diarrhea (Sabin et al., 1944). Toscana is another phlebovirus of medical importance that is one of the leading causes of meningitis in Italy and other neighboring European countries (Charrel et al., 2005; Echevarria et al., 2003; Peyrefitte et al., 2005). Punta Toro virus (PTV), a New World phlebovirus, causes an acute febrile illness that is generally self-limiting (Anderson et al., 1990). The most significant phleboviral pathogen is Rift Valley fever virus (RVFV). Epizootic outbreaks in domesticated ungulates can have devastating economic effects (Gerdes, 2002). In humans, RVFV infection can be quite severe in the form of viral hemorrhagic fever and encephalitis (Alrajhi et al., 2004; Peters and Meegan, 1981). Recent outbreaks in sub-Saharan Africa, Madagascar, Yemen, Egypt and Saudi Arabia have resulted in considerable morbidity and mortality (Centers for Disease Control and Prevention, 1994, 2000a,b, 2007; Morvan et al., 1991). Reflecting the concern of public health officials, RVFV has been classified as a "Category A" pathogen by the National Institute of Allergy and Infectious

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Diseases (NIAID, 2002), and has received dual "Select Agent" status by the Department of Health and Human Services and the United States Department of Agriculture.

Interferon (IFN) alfacon-1, trade name Infergen®, is a bio-optimized recombinant IFN-α that contains the most frequently occurring amino acids present among the non-allelic subtypes, of which there are at least 13 (Roberts et al., 1998). Compared to the naturally occurring IFN-α proteins, IFN alfacon-1 has demonstrated enhanced in vitro antiviral activity (Blatt et al., 1996). It is most commonly used for the treatment of hepatitis C virus infections in combination with the broad-spectrum antiviral, ribavirin (Sjogren et al., 2005; Tong et al., 1997; Witthoeft et al., 2007). Although IFN alfacon-1 activity is lacking in mice, we and others have previously demonstrated varying degrees of efficacy produced by treatment of acute viral diseases in different hamster infection models (Fish et al., 1985, 1986; Gowen et al., 2005; Julander et al., 2007; Morrey et al., 2004a,b). Collectively, these studies have indicated that the recombinant IFN alfacon-1 designed through consensus sequences present in human IFN-α proteins is reactive in the hamster system. While IFN alfacon-1 activity is likely to be considerably more potent in humans, activity in hamsters facilitates pre-clinical development for the treatment of human viral infections modeled in hamsters.

Although it is likely that all phleboviruses encode a type I IFN antagonist to subdue antiviral defenses in their maintenance hosts, thus allowing for sufficient replication and successful transmission, effective treatment of RVFV infection in rhesus monkeys with human INF-α has been previously reported (Morrill et al., 1989). In that study, Morrill and colleagues treated monkeys 24 h prior to or 6 h after infection. Since the early presence of type I IFN is associated with lack of clinical illness and survival in the rhesus model (Morrill et al., 1990), it is likely that efficacy would be reduced with delayed administration of IFN therapy. Further evidence supporting the need for rapid induction of type I IFN for the effective control of acute phleboviral infection and disease was recently shown in the hamster PTV infection model (Perrone et al., 2007). The PTV Adames strain is highly lethal in hamsters, while the Balliet strain of the virus produces a mild self-limiting infection (Anderson et al., 1990). The nonstructural protein encoded by the S segment of the Adames strain was found to disrupt type I IFN induction to a greater degree than that of the Balliet strain, likely contributing to the observed Adames strain lethality and its initial suppression of serum type I IFN levels following infection (Perrone et al., 2007). Thus, the treatment of patients having advanced RVFV infections with IFN-α may not provide much benefit. On the other hand, the encouraging data in rhesus monkeys would support the use of IFN-α in the event of accidental laboratory infection or possibly as a prophylactic measure for high-risk individuals during a severe epidemic.

IFN alfacon-1 has not been previously evaluated for the treatment of phleboviral infections. Here we investigated the prophylactic and therapeutic antiviral activity of IFN alfacon-1 against PTV infection in hamsters, as a model for severe RVFV pathogenesis and disease in humans. The ability of IFN alfacon-1 to protect animals from death and mitigate viral burden and liver disease was examined. Moreover, the effects of handling and stress associated with treatment of infection were also investigated.

2. Materials and methods

2.1. Cells and animals

The monkey kidney cell lines Vero 76 and LLC-MK_2 were purchased from American Type Culture Collection (ATCC; Manassas, VA) and maintained in minimal essential medium (MEM) supplemented with 0.18% $NaHCO_3$ and 10% fetal bovine serum (FBS; Hyclone, Logan, UT). Female 7–8-week-old golden Syrian hamsters were obtained from Charles River Laboratories (Wilmington, MA) and acclimated for 2–3 days prior to use. Animal procedures complied with USDA guidelines and were approved by the Utah State University Institutional Animal Care and Use Committee.

2.2. Viruses

PTV, Adames strain, was obtained from Dr. Dominique Pifat of the U.S. Army Medical Research Institute for Infectious Diseases, Ft. Detrick (Frederick, MD). The virus used for cell-based studies was from a stock prepared following four passages of the original virus through LLC-MK_2 cells. This virus was inoculated into hamsters for the production of a high titer preparation derived from pooled liver homogenates, and this stock was used for the in vivo challenge studies. The RVFV vaccine strain, MP-12, was provided by Dr. Robert Tesh (World Reference Center for Emerging Viruses and Arboviruses, University of Texas Medical Branch, Galveston, TX). The RVFV stock was prepared from virus passaged twice in Vero 76 cells.

2.3. Test materials

IFN alfacon-1 (trade name Infergen®), lot number A014896, was provided by Intermune, Inc. (Brisbane, CA) and had a reported activity of 1×10^9 units/mg. The material was at a concentration of 30 μg/ml and was further diluted to the appropriate concentrations with sterile saline. Ribavirin was supplied by ICN Pharmaceuticals Inc. (Costa Mesa, CA). Both drugs were prepared in MEM for cell culture experiments and in sterile saline solution for in vivo delivery.

2.4. Cell-based antiviral testing

Predetermined amounts of virus that would visually yield 80–95% CPE by day 6 in preliminary virus titration experiments were prepared in culture medium containing 2% FBS. Vero 76 cells plated in 96-well microtiter plates were exposed to one-half $\log_{10}$ dilutions of IFN alfacon-1 for 10–15 h prior to the addition of virus. Ribavirin was added to test wells at the time of infection. For toxicity determinations, drugs were incubated with cells in the absence of viral challenge. Plates were incubated at 37 °C, 5% CO_2, until virus-infected control wells presented with >80% cytopathic effect (CPE) by visual analysis, at which time plates were scored visually for CPE and toxic-