



Hantavirus Infections—Treatment and Prevention

Kalpa Dheersekara, BSc¹
Saranga Sumathipala, MD²
Rohitha Muthugala, MD^{1,*}

Address

¹Department of Virology, National Hospital Kandy, Kandy, Sri Lanka
Email: rohithavm@yahoo.com

²Department of Virology, Teaching Hospital Anuradhapura, Anuradhapura, Sri Lanka

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Abstract

Purpose of review Hantavirus infection is an emerging zoonosis and there are two main clinical presentations, hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS). Although Hantavirus infections have a worldwide distribution with a high mortality rate, a safe and effective vaccine or an antiviral drug against the Hantavirus disease is yet to be available. This review summarizes all the efforts undertaken to develop medical countermeasures in vitro, in vivo, and human clinical trials against Hantavirus infections.

Recent findings Multiple antivirals are shown to be effective with limited evidence and recent studies on immunotherapy were not very conclusive. There are multiple vaccine candidates with evidence of conferring long protective immunity against Hantaviruses. Some of these had been already trialed on humans.

Summary At present, severe HPS or HFRS case management is purely based on supportive treatments, often in an intensive care unit. Rodent control and public health education and promotion play a major role in preventing Hantavirus infection.

Introduction

Hantavirus is an enveloped virus with a negative sense, tri-segmented RNA genome, and belongs to the family *Bunyaviridae* which includes more than

twenty different Hantavirus species [1, 2]. The three genome segments named small (S), medium (M), and large (L) encode for the nucleoprotein, the

glycoprotein (Gn and Gc), and the viral RNA-dependent RNA polymerase respectively [3–5]. These viruses are zoonoses and are responsible for two human disease entities namely hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS) or Hantavirus cardiopulmonary syndrome (HCPS). These two disorders are associated with fever with acute thrombocytopenia and changes in vascular permeability, and both forms may have renal and/or pulmonary symptoms [6–8]. Transmission of the infection to humans occurs by inhalation of aerosols contaminated with virus-containing rodent excreta. The lack of apparent disease in natural hosts and lack of suitable animal models are significant obstacles in understanding the pathogenesis of HFRS and HPS [9]. Following inhalation, cellular entry of pathogenic Hantaviruses appears to be mediated by $\beta 3$ integrin receptors, which are present on the surfaces of platelets, endothelial cells, and macrophages [10]. These cells allow virus replication which induces intense immune activation mediated by macrophages and CD8 T cells [11]. Activated macrophages secrete proinflammatory cytokines such as TNF- α , IL-1, and IL-6 which ultimately lead to increase vascular permeability resulting in fluid leakage into body cavities causing circulatory and respiratory failure [6]. Immune-mediated damage to vascular endothelium and platelets leads to hemorrhage, which is a key feature of Hantavirus disease in humans.

Old World Hantaviruses, which includes Hantaan virus (HNTV), Dobrava virus (DOBV), Puumala virus (PUUV), and Seoul virus (SEOV), cause HFRS with a <1–10% fatality rate in Asia and Europe and New-world Hantaviruses, which includes Sin Nombre virus (SNV) and Andes virus

(ANDV), cause HPS with a fatality rate of 30–40% in North and South Americas [12–15]. In contrast to other Hantaviruses, SEOV has a worldwide distribution due to the dispersion of its rodent reservoir, *Rattus norvegicus*, all over the world [16]. Approximately, 40,000–60,000 HFRS cases have been reported annually in Asia with HTNV and SEOV, which 90% of these cases come from China [17]. The majority of HFRS cases in Europe were due to PUUV causing mild disease while DOBV was being responsible for the severe hemorrhagic manifestations [13, 14]. Since the first outbreak of HPS in 1993, thousands of HPS cases have been reported in a sporadic fashion throughout North America with 624 HPS cases during 1993–2013 in the USA and with 109 cases during 1994–2014 in Canada. About 4000 HPS cases have been reported from South America with 103 cases during 1995–2012 in southern Chile, 519 cases during 2009–2017 in Argentina, and 884 reported cases during 1993–2007 in Brazil. Human to human transmission of ANDV-HPS reported from Chile and Argentina [18, 19].

Hantavirus infections are distributed worldwide with a high mortality rate and human to human transmission is also possible as mentioned above, highlighting the importance of medical countermeasures for Hantavirus infection prevention and treatments. Although a large number of studies had taken place to identify and develop antiviral therapies and vaccines to prevent and treat Hantavirus infection, as of today there is not a single WHO or FDA approved vaccine or therapy available for patients. This review summarizes all the efforts which had been undertaken to develop therapies and vaccines for Hantavirus infections so far.

Treatment

To date, there are no US Food and Drug Administration–approved antiviral drugs available for use against HFRS or HPS. Therefore, the management of severe cases is purely based on supportive care. Maintaining fluid and electrolyte balance is very important in these patients. HFRS patients with severe renal insufficiency may need extracorporeal blood purification (dialysis treatment). In HCPS, mechanical ventilation or even extracorporeal membrane oxygenation may be required.

Additional to supportive care, there had been trials of antiviral and immunotherapies done against HFRS and HPS. They are as follows:

Ribavirin

Effectiveness of ribavirin against some Hantaviruses had been proved in vitro and to some extent in vivo. Efficacy of ribavirin therapy against HFRS has been evaluated using a Hantaan virus-infected suckling mouse model with various doses of ribavirin. Treatment with 50 mg of ribavirin/kg per day beginning on day 10 of the viral challenge demonstrated a higher survival rate compared with placebo controls in mice [20]. Ribavirin efficacy against Andes virus-related HPS has been shown in vitro using Vero E6 cells and in vivo using a lethal hamster HPS model. In hamsters, treatment with as little as 5 mg/kg per day was effective in preventing lethal HPS disease when therapy was administered via intraperitoneal injection from day 01 through day 10 post-infection [21]. Ribavirin remained 100% protective when administered via intraperitoneal injections up to 03 days following infection [21]. Also, another study has shown 100 mg/kg and 50 mg/kg concentrations of ribavirin prevented HPS in hamsters without toxicity, and administration of ribavirin at 14 days post-infection also provided a significant level of protection against lethal HPS [22]. These data provide in vivo evidence supporting the potential use of ribavirin as a post-exposure treatment to prevent HPS caused by ANDV. However, two clinical trials involving the treatment of HPS using ribavirin have shown that ribavirin was ineffective for patients having HPS progressed to the cardiopulmonary phase [23, 24].

A double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin has been conducted in the People's Republic of China with 242 HFRS patients. Patients were treated with a loading dose of 33 mg/kg, followed by a dose of 16 mg/kg every 6 h for 4 days, and 8 mg/kg every 8 h for the subsequent 3 days [25]. Mortality was significantly reduced (sevenfold decrease in risk) among ribavirin-treated patients. Conversely, one open-labeled clinical trial, conducted in the European part of Russia, showed insufficient efficacy of IV ribavirin treatment for HFRS caused by PUUV [26].

Most of the completed clinical trials have shown anemia which is reversible after completion of ribavirin therapy [25, 26]. Additionally, some patients have developed hyperbilirubinemia, sinus bradycardia, and rashes [26].

Favipiravir

Favipiravir (T-705) potently inhibits SNV and ANDV in vitro [27]. For both viruses, the 90% effective concentration was estimated at ≤ 5 $\mu\text{g/ml}$. In the lethal ANDV hamster model, the daily administration of oral T-705 at 50 or 100 mg/kg significantly improved the survival rate. Oral T-705 therapy remained protective against HPS when treatment was initiated prior to the onset of viremia. Using a hamster-adapted SNV, daily administration of oral T-705 significantly reduced the detection of SNV RNA and antigen in tissue specimens, suggesting that the compound would also be effective against HPS. T-705 is well tolerated in humans, with no significant adverse effects noted in human clinical trials. Supporting the low toxicity of this compound, the 50% lethal dose (LD_{50}) of T-705 is at least 6 times greater than that of ribavirin in hamsters [28].

Lactoferrin

Efficacy of lactoferrin, an iron-binding glycoprotein, against Hantavirus has been demonstrated *in vitro* and *in vivo* [29, 30]. The result of both studies has shown the antiviral activity of lactoferrin against Hantavirus by inhibition of virus adsorption to the cells. And also complete inhibition of foci formation with lactoferrin and ribavirin *in vitro* demonstrated the synergetic effect of both drugs. *In vivo* lactoferrin pre-treatment was evaluated by administration of 40 and 160 mg/kg concentrations prior to Hantavirus viral challenge in suckling mice and improved survival rates were demonstrated to 15% and 70% with a single administration and 85% and 94% with double administration, respectively.

Vandetanib

Increased vascular permeability is an important determinant in severe disease progression of viral hemorrhagic fevers [31]. Endothelial cells infected with ANDV induce the production of vascular endothelial growth factor (VEGF) and VEGF activates VEGFR2 receptors on endothelial cells. VEGFR2 activation induces the internalization of vascular endothelial cadherin from adherent junctions and increases paracellular permeability [32–34]. Moreover, elevated circulating levels of VEGF have been seen in patients with severe HPS indicating the VEGF as the potential key factor in the pathogenesis of HPS [33]. Also, increased levels of proinflammatory cytokines and VEGF-A in response to the ANDV challenge have been shown in a 3D human lung model [35]. Vandetanib is a tyrosine kinase inhibitor targeting VEGF-receptor 2 activation and the ability to block VEGFR-2 phosphorylation and VE-cadherin degradation by vandetanib has been demonstrated *in vitro* [36]. Furthermore, delayed lethality and increased total survival by 23% has shown in ANDV/hamsters models with 10, 25, and 50 mg/kg/day of Vandetanib starting 05 days before ANDV challenge. Unfortunately, potentially serious adverse events including dermatologic reactions, hypertension, and other cardiopulmonary effects in large-scale human trials have shown with many of the early VEGFR-2 tyrosine kinase inhibitors, including Vandetanib, that were developed for cancer therapy [37].

Immunotherapy

Although currently, no specific treatment has been shown to be effective against Hantaviruses causing HPS, several studies have demonstrated that neutralizing antibodies can inhibit HPS *in vivo*. Inoculation with recombinant DNA vaccines (ANDV M/SNV M) and passive transfusion of polyclonal serum from geese, ducks, and rabbits have protected hamsters from HPS [38–41]. In Chile, an open trial has been conducted to evaluate the efficacy and safety of human immune sera as a treatment option for HPS [42]. Thirty-two suspected and confirmed HPS patients were treated via intravenous infusion at an ANDV neutralizing antibodies dose of 5000 U/kg. Results have shown a borderline statistical significance when compared with the case-fatality rates of 32% in the rest of the country during the study period.

Two recently developed recombinant monoclonal antibodies have protected hamsters from lethal ANDV-HPS [43]. For this, 27 ANDV convalescent HCPS sera have been screened and one source subject was selected with high antibody titers. Recombinant monoclonal antibodies have been

developed from isolated memory B cells, using recombinant DNA technology. The resulting monoclonal antibody candidates, JL16 and MIB22, had been shown to effectively neutralize ANDV *in vitro*.

ETAR

1-β-D-Ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR) is a nucleoside analog which is active against HTNV and ANDV Hantavirus *in vitro*. Intraperitoneally delivered ETAR has offered protection to suckling mice at 10 days after being challenged with HTNV with around 25% survival at 12.5 and 25 mg/kg of ETAR [44].

Corticosteroids

A study has been done using a parallel-group, placebo-controlled clinical trial in Chile to see the efficacy of intravenous methylprednisolone for HPS infection [45]. It concludes as there is no significant difference in mortality between treatment groups was observed and it did not provide significant clinical benefit to patients. Moreover, results do not support the use of methylprednisolone for HPS.

Prevention

People in contact with rodents or their excreta are the risk group for Hantavirus infection. Therefore, rodent control in households and in other areas where human activities are involved is considered the most important step in the prevention of the disease. Rodent controlling should include the removal of rodent food sources inside and around the home, measures to prevent rodents from entering the home, use of rodent traps, and the removal of possible nesting sites around the home [46]. Ventilation of the rooms before entering, use of rubber gloves and disinfectants, use of respirators to avoid breathing in contaminated particles while cleaning up potentially rodent-infested areas and rooms are important to reduce the risk of exposure to rodent excreta [6]. Additionally, for the specific prevention of human infections, mainly in risk groups, Hantavirus vaccines are necessary.

First-generation vaccines

Inactivated HFRS vaccines in China and Korea

Both cell culture and rodent-brain-derived vaccines have been developed and tested in humans in China and Korea. During 1950–2007, more than 1.5 million cases of HFRS in humans and more than 45,000 deaths (3%) were reported in China. However, HFRS incidence and the mortality rate have significantly decreased with the implementation of comprehensive preventive measures, including vaccination. Since 1995, HTNV and SEOV inactivated vaccines have been used in areas where HFRS is highly endemic. Purified bivalent vaccines for HTNV and SEOV inactivated with formaldehyde, cultured in Vero cells, are being in use since 2003 [47]. The levels and the positive rates of HTNV-NP-specific IgM and IgG antibodies, as well as HTNV neutralizing antibodies, were significantly increased in the serum of the vaccinated individuals.

The positive rates and levels of HTNV-NP-specific IgG and HTNV neutralizing antibody are shown to reach their highest values at 3 months after vaccination and high seropositive rates were sustained up to 33 months after vaccination [48].

A formalin-inactivated HATN vaccine (Hantavax) has been widely used for HFRS by South Korea. Sero-conversion and high specific antibody titers in humans were shown with a recommended vaccination strategy of three doses (0 day, 01 month, and 12 months). Less than 50% of the sampled population produced neutralizing antibodies following the booster dose after 12 months [49]. A phase III, multi-center clinical trial was undertaken to evaluate the immunogenicity and safety of Hantavax (three-dose schedule at 0, 1, and 13 months) among healthy adults [50]. Hantavax showed a booster effect and immunogenicity lasting 2 years with a three-dose schedule. The neutralizing antibody response was quite poor with two primary doses, so an early booster vaccination at 2–6 months might be warranted to provide timely protection to high-risk subjects. However, a case-control study conducted in the Korean army had not shown statistically significant effectiveness even after the three-dose vaccination [51].

Second-generation vaccines

Virus-like particle vaccine

Although inactivated vaccines for HFRS have decreased the number of cases per year in China and Korea for years, it is less sufficient for long-term antibody levels; loss of effective cell-mediated immunity and frequent immunization are recommended. Therefore, there has been arising interest in developing vaccine strategies that are more capable of inducing more broadly and long-lasting immunity against Hantavirus. Virus-like particle vaccine has been induced safe, long-lasting, and high titer antibody levels in humans for some viruses [52]. In China, HTNV virus-like particles (VLPs) decorated with CD40L or GM-CSF had been constructed using the HTNV M segment and CD40L/GM-CSF gene, co-transfected with a vector containing S segment into dihydrofolate reductase Chinese hamster ovary cells (dhfr-CHO). CD40L or GM-CSF-anchored HTNV VLP showed enhanced activation of macrophages and dendritic cells in vitro [53, 54]. These HTNV VLPs have provided stable, long-term protection with a high titer of neutralizing antibody in mice 6 months after immunization and HTNV-specific cellular immune responses via higher expression of IFN- γ and CTL responses [54]. CD40L or GM-CSF decorated VLPs induced humoral and cellular immunity were greater than undecorated VLPs or inactivated HTNV vaccines in mice [53, 54].

European researchers have shown the high protection against PUUV challenge in the bank vole model using a chimeric hepatitis B virus core particle carrying a 45 amino acid fragment of PUU strain CG18–20 N inserted in the c/e1 region [55, 56]. Immunizations with VLPs carrying amino acids 75–119 of PUU strain CG18–20 at the C-terminus of HBV core has been demonstrated the presence of a second, minor protective region in the nucleocapsid protein.

HBV core particles carrying the N terminal 120 amino acids of the nucleocapsid protein of the Dobrava, Hantaan, or Puumala have been shown to be highly immunogenic with or without adjuvant in B ALB/c and C57BL/6 mice

[57, 58]. The induced nucleocapsid specific antibodies represented all IgG subclasses and strongly cross-reactive. And also, pre-existing core-specific antibodies did not abrogate the induction of an N-specific immune response.

Recombinant proteins

There is enough evidence that both the membrane glycoproteins (Gn and Gc) and the nucleocapsid protein have strong antigenicity and can induce protection against Hantaviruses. Moreover, several techniques have been proposed for the development of Hantavirus recombinant protein vaccines [59–64]. Baculovirus recombinants expressing both Gn and Gc have induced higher antibody titer responses than those expressing only Gn or Gc [59]. And also, baculovirus recombinants expressing only nucleocapsid protein or Gn and Gc combination have given full protection in hamsters from a challenge. Amino terminus has identified as the main antigenic domain, harboring six out of seven B cell epitopes in the nucleocapsid protein of PUUV, recognized by infected bank vole monoclonal antibodies.

Using adjuvant has enhanced the antigenicity and protective efficacy of the Hantavirus recombinant proteins. Freund's adjuvant (not for use in humans), alum, aluminum hydroxide, or a genetically fused or complexed protein, such as the outer membrane protein A of *Klebsiella pneumoniae* (rP40), the human IL-2 gene, or the heat-shock protein have been used as an adjuvant. The nucleocapsid protein is more conserved among different Hantavirus species. Therefore, the nucleocapsid protein induces highly cross-reactive antibody responses.

Third-generation vaccines

Recombinant vector vaccine

Recombinant vector vaccines have shown to be effective in the prevention of Hantavirus infection in animals [65–68]. A double-recombinant molecular vaccine has been prepared by inserting the cDNA representing the M and S genome segments of HTNV into the vaccinia virus [69]. This vaccinia vector recombinant vaccine was effective in protecting hamsters from challenge with Hantaan and Seoul viruses but not with PUUV [65]. Moreover, this vaccine has been evaluated in phase I and phase II clinical trials [70]. According to the phase I results, neutralizing antibody titers have been increased to both vaccinia virus and HTNV with the second inoculation. Comparing two routes of vaccination has shown that scarification effectively induced neutralizing antibodies in vaccinia virus-naïve volunteers but that subcutaneous inoculation was superior to scarification in vaccinia virus-immune individuals. Results of the phase II trial demonstrated that neutralizing antibodies to HTNV were detected in 72% of vaccinia virus-naïve volunteers and only in 26% of the vaccinia virus-immune volunteers. Consequently, this vaccine has not been pursued.

Non-replicating adenovirus vector, expressing ANDV N, Gc, Gn, or Gn + Gc elicited a strong immune response that protected hamsters with lethal ANDV infection and elicited strong cytotoxic T lymphocyte responses in mice [67]. However, the problem of pre-existing immunity to adenovirus type 5 remains substantial, highlighting the need for the development of vectors using less common adenovirus serotypes.

Vesicular stomatitis virus (VSV) pseudotype expressing HTNV Gn and Gc was immunogenic and protective with the third immunization in the mice challenged with HTNV [66]. Replication competent recombinant vesicular stomatitis virus (VSV) expressing ANDV Gn and Gc precursor demonstrated the potential for the use as a fast-acting, pre and post-exposure efficacy against lethal ANDV challenge in the Syrian hamster model [68]. However, a booster vaccination schedule might be required to provide long-term immunity [71].

Nucleic acid–based molecular vaccine

DNA vaccines containing either the M or S gene segment of SEOV has constructed by using three different vectors, naked DNA expression vector (pWRG7077), DNA-based Sindbis replicon (pSIN2.5), and packaged Sindbis replicon vectors (pSINrep5) [72, 73]. Protection was associated with the M segment vaccines and gene gun inoculation was superior to the needle inoculation. Moreover, hamsters were protected from both SEOV and HTNV infections. Consequently, another DNA vaccine was developed expressing the HTNV M gene segment. This vaccine was given sterilizing immunity against HTNV, SEOV, and DOBV in hamsters and elicited very high levels of neutralizing antibodies in monkeys [74]. Another three different M gene segment DNA vaccines for ANDV, PUUV, and SNV were developed. PUUV DNA vaccine elicited high-titer neutralizing antibodies in hamsters and nonhuman primates and protected hamsters against infection with PUUV and ANDV but not against other HFRS-associated Hantaviruses [75]. And also, ANDV and SNV DNA vaccines elicited high titers of neutralizing antibodies in monkeys and rabbits, respectively [40, 76]. Furthermore, rabbits were vaccinated with HPS mix (ANDV and SNV plasmids), or HFRS mix (HTNV and PUUV plasmids), or HPS/HFRS mix (all four plasmids) to test the possibility of producing a pan-Hantavirus vaccine. The HPS/HFRS mix elicited neutralizing antibodies against all four viruses. However, protection following vaccination with the multivalent Hantavirus vaccines was not tested.

Two (phase I) clinical trials were conducted to test the efficacy and the safety of HTNV and PUUV M segment DNA viruses according to the vaccine delivery technology [77, 78]. Vaccines were considered safe with no serious adverse effect for human use in both trials. When vaccines delivered by particle mediated epidermal delivery (PMED), 30% and 44% of individuals developed neutralizing antibodies to HTNV or PUUV, respectively in single vaccine groups, and 56% of the volunteers developed neutralizing antibodies to one or both viruses in the combined vaccine group. However, the overall sero-conversion rate (below 50%) with PMED delivery was too low for further development. With the intramuscular electroporation delivery method, 56% and 78% of individuals developed neutralizing antibodies to HTNV or PUUV, respectively in single vaccine groups and 78% of the volunteers developed neutralizing antibodies to PUUV in the combined vaccine group. According to these results, it has clearly demonstrated that the immunogenicity can be enhanced with the advances of the delivery technology.

Two different DNA vaccines were developed targeting the HTNV Gn or HTNV Gc fused with lysosome-associated membrane protein 1 (LAMP1). LAMP 1 alters antigen-presenting pathway and activated the CD4+ T cells which can elicit strong humoral, cellular, and long-term immune response [79, 80].

Both vaccines were demonstrated in the long-term immune responses in vivo [80•, 81].

Conclusion

Hantavirus infections do not have any regulator approved therapeutic options. However, multiple antivirals are shown to be effective for these pathogens with limited evidence. Most of this evidence comes from in vitro studies and from animal models. Therefore, human trials are required to gain an unbiased knowledge on clinical efficacy and safety of these therapies. Antivirals such as ribavirin which has shown evidence in limited human trials are readily available commercially. These could be considered as off-label therapeutic options in certain situations or under compassionate use.

There are multiple vaccine candidates with evidence of conferring long protective immunity against Hantaviruses. Some of these had been already trialed on humans by some nations. Larger regulatory bodies such as FDA are yet to approve and WHO is yet to pre-qualify a vaccine for a Hantavirus. More clinical trial data are required for vaccines as for therapeutics to ensure that they can be made available and used at a wider scale.

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