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MINIREVIEW

Antiviral therapy and prevention against hantavirus infections

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Summary. – Hantaviruses are emerging zoonoses hosted by small mammals. In humans, they cause two diseases. Hemorrhagic fever with renal syndrome is mainly caused by Dobrava-Belgrade virus, Puumala virus, Seoul virus and Hantaan virus in Asia and Europe. On the other hand, the most important causes of hantavirus cardiopulmonary syndrome are Sin Nombre virus and Andes virus in Americas. Ribavirin yet remains the only licensed drug against the hantavirus infections, but its sufficient antiviral activity remains an issue under discussion. There are still no available vaccines against hantaviruses except of some inactivated virus vaccines licensed only in East-Asian countries. Some of the vaccines are under development in pre-clinical stages. The review discuses about specific compounds with approved antiviral activity against hantaviruses. Other approaches such as development of vaccines, are compiled as well.

Keywords: hantavirus; HFRS; HCPS; antiviral drugs; vaccines

Contents:

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Abbreviations: ANDV = Andes virus; DOBV = Dobrava-Belgrade virus; ETAR = 1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole; HCPS = hantavirus cardiopulmonary syndrome; HFRS = hemorrhagic fever with renal syndrome; hpi = hours post infection; HTNV = Hantaan virus; LF = Lactoferrin; PUUV = Puumala virus; RBV = Ribavirin; SEOV = Seoul virus; SNV = Sin Nombre virus; VLPs = virus-like particles

1. Introduction

Hantaviruses (the family *Bunyaviridae*, the genus *Hantavirus*) have been discovered more than 35 years ago. They are considered as emerging zoonoses due to their significance as human pathogens and their increasing repetitive appearance during outbreaks. Hantaviruses are causative agents of two human diseases: hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe and hantavirus cardiopulmonary

syndrome (HCPS) caused by hantavirus species circulating in Americas.

Hantaviruses form enveloped virus particles which contain negative-sense single-stranded RNA genome segmented into the small (S), medium (M), and large (L) segments encoding a nucleocapsid (N) protein, a glycoprotein precursor (GPC), and the viral RNA-dependent RNA polymerase (RdRp), respectively (Schmaljohn and Nichol, 2007).

Hantaviruses are asymptomatically harbored by their reservoir small mammal hosts, mainly rodents. Over the last decade, it became obvious that besides rodents, hantaviruses are carried also by small insectivorous mammals such as shrews, moles, and bats which even seem to be the ancestral hantavirus hosts (Witkowski *et al.*, 2016). Hantaviruses are transmitted by aerosolized excreta (urine, saliva and feces) of their reservoir hosts. A rare way of infection is by bite of the infected animal (Douron *et al.*, 1984). The observation of Andes virus (ANDV) infection in Syrian hamsters indicates that transmission by intragastric administration is also possible, what can mean a potential risk of hantavirus infection by contaminated food (Hooper *et al.*, 2008). Hantaviruses are the only genus within the family *Bunyaviridae* which is not transmitted by arthropod vectors (Yu and Tesh, 2014).

The most important hantavirus species that cause HFRS in Eurasia are Hantaan virus (HTNV), Puumala virus (PUUV), and Dobrava-Belgrade virus (DOBV) while ANDV and Sin Nombre virus (SNV) are the main causative agents of HCPS in Americas (Table 1) (Klempa *et al.*, 2013). Meanwhile, many other species of hantaviruses have been found, also in Africa, where they could represent a public health threat, as well. The pathogenic and epidemiological potential of the African hantaviruses has not been fully discovered, yet (Klempa *et al.*, 2012). Both, HFRS and HCPS, are acute febrile infections. The incubation time before onset of first symptoms is usually 2–3 weeks, but there was also reported a range between 1–6 weeks. Common symptoms for HFRS and HCPS in the early phase (3–5 days) of disease are fever, myalgia, malaise, headache, backache and abdominal pain. Nausea and diarrhea appears also often. Hypotension occurs in the next phases (2–7 days) which means also a risk of cardiac failure and death. In HCPS, lung edema appears and leads to lung failure. In contrary, next phases of HFRS are accompanied by renal failure (Krüger *et al.,* 2011). Approximately 150,000 to 200,000 of HFRS cases are hospitalized every year. This number varies depending on epidemic year. The case fatality rate (CFR) of HFRS varies from <1% to 12%. There are about 200 cases of HCPS per year with CFR up to 40% in Americas (Bi *et al.*, 2008; Makary *et al.*, 2010; Hjertqvist *et al.*, 2010; Macneil *et al.*, 2011).

Therapy of hantavirus diseases is usually based on the supportive care such as hemodialysis in HFRS (Bren *et al.*, 1996), mechanical ventilation, extracorporeal membrane oxygenation (Guilfoyle and Macnab, 2008; Wernly *et al.*, 2011) and hemofiltration in HCPS (Bugedo *et al.*, 2016), and/or shock therapy in both of them. Ribavirin is still the only established drug with approved *in vitro* and *in vivo* effects against hantavirus replication (Ogg *et al.*, 2013; Krüger *et al.*, 2011; Westover *et al.* 2016). In this review, we summarize current approaches to cure and prevent the hantavirus diseases including those not directly targeting the virus but reducing the pathogenesis of the hantavirus infection.

2. Virus-targeting antivirals

2.1 Ribavirin

Ribavirin (RBV) is a broad-spectrum chemical compound with efficacy against many DNA and RNA viruses, including hantaviruses *in vitro* and *in vivo* (Sidwell *et al.*, 1972; Graci *et*

Table 1. The most important hantaviruses causing hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS)

Virus species	Abbreviation	Disease	Reservoir host	Geographic distribution	Case fatality rate (%)
Hantaan virus[a]	HTNV	HFRS	*Apodemus agrarius*	Asia	10–15
Dobrava-Belgrade virus[b]	DOBV	HFRS	*Apodemus agrarius* *Apodemus flavicollis* *Apodemus ponticus*	Europe	up to 12
Seoul virus[c]	SEOV	HFRS	*Rattus rattus* *Rattus norvegicus*	worldwide	1–2
Puumala virus[c]	PUUV	HFRS	*Myodes glareolus*	Europe	0.1–0.4
Sin Nombre virus[d]	SNV	HCPS	*Peromyscus maniculatus*	USA, Canada	30–50
Andes virus[d]	ANDV	HCPS	*Oligoryzomas longicaudatus*	Argentina, Chile, Uruguay	30–50

[a]Zeier *et al.*, 2005; Hooper *et al.*, 2006; [b]Rizzoli *et al.*, 2015; [c]Goeijenbier *et al.*, 2015; [d]Jonsson *et al.*, 2008.

al., 2006). The mechanisms of antiviral activity are based on its ability to inhibit inosine monophosphate dehydrogenase, a crucial enzyme responsible for the synthesis of GTP *de novo*. Other targets for its antiviral effect have been described, such as capping, translational efficiency of viral mRNA, and a suppressive effect on the viral polymerase activity (Chung *et al.*, 2013). Against hantaviruses, the mechanism of RBV seems to be more likely virus-unspecific. RBV was proved also as a potent mutagen of viral RNA (Crotty *et al.*, 2001, 2002; Jonsson *et al.*, 2005; Chung *et al.*, 2007, 2013).

RBV was reported to play a role in the immune response by down-regulation of interleukin 10 (IL-10)-producing Treg 1 cells, which could inhibit the conversion of $CD4^+$ $CD25^-$ $FOXP3^-$ naive T cells into $CD4^+$ $CD25^+$ $FOXP3^+$ adaptive Treg cells to maintain Th1 cell activity. However, the RBV-induced immune response against hantavirus infection is not yet fully discovered as well as the mutagenesis induced by RBV and its influence on next generations of virions (Kobyashi *et al.*, 2012).

The antiviral activity of RBV against HFRS and HCPS associated hantaviruses was tested *in vivo* and *in vitro*, as well (Huggins *et al.*, 1986; Chung *et al.*, 2013; Safronetz *et al.*, 2011). RBV-treated suckling mice infected by HTNV showed significantly higher survival rate than the placebo control group (Huggins *et al.*, 1986). A double-blind placebo-controlled test of HFRS Chinese patients resulted in sevenfold lower morbidity and fatal ending in RBV-treated group (Huggins *et al.*, 1991). The rates of oliguria and renal insufficiency are lower after the treatment by RBV, which increases the survival rate. In contrary, RBV used against HCPS seems to be more ineffective (Chapman *et al.*, 1999; Mertz *et al.*, 2004; Chung *et al.*, 2013). Interestingly, RBV

Table 2. List of drugs which could be used against HFRS and HCPS

Drug	Target	Application	Commonly reported adverse side-effects	
			More common	Less common
Ribavirin[a]	RdRp	oral (capsule, solution, tablet), intravenous solution, inhalation powder for solution	hemolytic anemia, decreased hemoglobin, insomnia, dyspnea, lack of concentration, emotional lability and irritability, nervousness teratogenicity	depressed mood, dry skin, feeling cold, muscle cramps and stiffness
Lactoferrin[b]	virus adsorption	oral solution	not observed	diarrhea, in very high doses: skin rash, loss of appetite, fatigue, chills, constipation
Favipiravir[c]	RdRp	oral tablet	not observed	not observed
ETAR[d]	RdRp	not in clinical use	undiscovered	undiscovered
Icatibant[a]	bradykinin B2 receptor	subcutaneous solution	bleeding, inflammation, burning, coldness, pain, redness, stinging, tingling at the injection site	dizziness fever
Methylprednisolone[a]	humoral immune response	oral tablet, intravenous solution	aggression, agitation, anxiety, blurred vision, dizziness, headache, irritability, mood changes, nervousness, irregular pulse, troubled breathing at rest pounding in the ears, weight gain	not observed

[a]Compiled from Drugs.com; data sources include Micromedex® (updated July 1st, 2016), Cerner Multum™ (updated July 7th, 2016), Wolters Kluwer™ (updated July 6th, 2016) and others (Edelson, 1991; Barry *et al.*, 1986; Robertson, 2008; Mertz *et al.*, 2004); Product infromations: Rebetol (ribavirin), Schering-plough Corporation; Virazole (ribavirin), ICN Pharmaceuticals Inc; Copegus (ribavirin), Roche Laboratories. [b]Compiled from WebMD.com; copyrighted data are provided by *Natural Medicines Comprehensive Database Consumer Version* (FDA, 2003; Harmsen *et al.*, 1995; Ishibashi *et al.*, 2005; Puddu *et al.*, 1998); [c]Arias *et al.*, 2014; [d]Chung *et al.*, 2008.

appears to be sufficiently active in treatment of HCPS caused by ANDV (Safronetz *et al.*, 2011).

RBV is associated with potentially serious side effects, such as anemia and the teratogenicity if used in pregnant women (Table 2). Some studies suggest that there is no significant difference in the frequency of adverse events (Mertz *et al.*, 2004). Severe anemia appears in about 10% of treated patients, therefore a monitoring of hemoglobin is required. In cases of anemia, the reduction of RBV doses is needed, but this can cause compromising of sustained virologic response. Anemia is most probably a consequence of RBV accumulation in erythrocytes due to straight unidirectional transport through the membranes. Nowadays, the only prevention of RBV-induced anemia is the concomitant administration of erythropoietin (Russmann *et al.*, 2006).

2.2 Lactoferrin

Lactoferrin (LF), an iron-binding glycoprotein, besides of antibacterial and antifungal effect was reported to have a broad antiviral activity (Bullen and Armstrong, 1978; Masson *et al.*, 1969; Yi *et al.*, 1997). It has been demonstrated that LF also inhibits hantavirus infection *in vitro* and *in vivo* (Murphy *et al.*, 2000, 2001).

The antiviral effects of LF against hantaviruses was compared with those of RBV in study which was performed on Vero E6 cells infected by Seoul virus (SEOV). Post infection administration of 100 μg/ml of RBV inhibited the number of foci by 97.5%. 400 μg/ml of LF reduced the number of foci by 85% in comparison with cells of the control group. In cells pretreated with LF, the number of foci initiated to increase from 24 h post infection (hpi). LF inhibited viral shedding at 24 hpi, but not after 48 hpi (Murphy *et al.*, 2001). Therefore, LF obviously inhibits an early phase of infection, most probably adsorption as indicated in another supportive study (Murphy *et al.*, 2000). Accordingly, the inhibition of hantavirus glycoprotein (G2) expression was observed. By 48 hpi, the expression of G2 was increased in both, the control and LF pretreated cells. The complete G2 inhibition was detected only in cells treated with the combination of LF/RBV from 12 hr on. The inhibition of adsorption theory is supported also by the fact that LF does not inhibit the expression of hantavirus G2 and N protein when the infection is just established in cells (Murphy *et al.*, 2001).

On the other hand, RBV actively inhibits viral protein expression within the cell and does not inhibit viral adsorption (Huggins *et al.*, 1984; Streeter *et al.*, 1973). RBV apparently inhibits viral transcription and reduces a massive release of virions from infected cells. Nevertheless, RBV solely is not able to eliminate the virus completely as well as LF. Both, RBV and LF gave significantly higher survival rates in test *in vivo*. RBV administered 1 hpi to mice at dose of 50 and 25 mg/kg gave 81.8 and 68.8% survival rates, respectively (Murphy *et al.*, 2001). These results are well experimentally supported by other studies, too (Huggins *et al.*, 1991). Lactoferrin administered with dose of 160 mg/kg to mice 1 day prior to hantavirus inoculation had a survival rate of 70%. Double administration of LF enhanced the survival rate. The 160 and 40 mg/kg double administration resulted in 94.1% and 85.7% survival rates, respectively. The difference between single and double administration of LF could be probably due to insufficient adsorption in the single dose or an accumulative effect of LF in the body from the two administrations. Another reason of this difference could be a certain period of time which is necessary for activation of immune system by LF. It has been demonstrated that LF enhances cytotoxic activities of monocytes and NK cells (Murphy *et al.*, 2001).

2.3 Favipiravir

Favipiravir (Avigan; T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is an antiviral drug selectively inhibiting the RNA-dependent RNA polymerase mainly of influenza virus. Its efficacy against the hantaviruses, Maporal virus, DOBV and Prospect Hill virus was also reported *in vivo* in mice and hamsters. Its activity *in vitro* against these hantaviruses was in the range of 5–30 μg/ml (32–191 μmol/l), as calculated by results of FFU reduction assays. (Gowen *et al.*, 2007, 2010; Buys *et al.*, 2011).

Favipiravir decreased detection of viral RNA and reduced infectious titers of SNV and ANDV *in vitro*. For both, the EC50 was calculated at ≤5 μg/ml (≤31.8 μmol/l). In hamsters infected with ANDV, favipiravir reached 100% of effectiveness at preventing lethal HCPS when hamsters were administrated with favipiravir on or before day 4 post exposure. In contrast, animals of the placebo group demonstrated breathing difficulties on day 6 or 7 post infection leading to severe respiratory distress with a fatal outcome by day 9 (Buys *et al.*, 2011; Safronetz *et al.*, 2013).

2.4 ETAR

1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR) is a novel, nucleoside analogue. ETAR as well as RBV is a 3-substituted 1,2,4,-triazole-β-riboside, but with altered steric and hydrogen bonding capacity. Its mechanism is based on inosine monophosphate dehydrogenase inhibition with reduction of GTP pools, which was combined with residual complementary activity possibly affecting the L protein (Kumarapperuma *et al.*, 2007; Goundry *et al.*, 2003).

The antiviral activity of ETAR against HTNV and ANDV as representatives of HFRS and HCPS was approved. The EC50 values for HTNV and ANDV according to FFU-reduction assay were 10 μmol/l and 4.4 μmol/l, respectively. ETAR was not toxic to Vero E6 cells up to a concentration of 880 μmol/l. Moreover, ETAR protected suckling mice from

HTNV infection at similar degree as RBV. The evaluation of ETAR in the suckling mice model infected by HTNV showed that the *in vivo* antiviral activity of ETAR at the 12.5 and 25 mg/kg doses was similar to that of 50 mg/kg RBV (Chung *et al*., 2008).

As shown previously, RBV is responsible for increased frequency of errors during replication of the HTNV leading to increased mutation frequency. Although ETAR is structurally similar to RBV, a comparison to the placebo-treated HTNV group showed no significant change in mutation frequency caused by ETAR. The metabolites of ETAR accumulate to lower concentrations in cells than the metabolites of RBV, which means that ETAR metabolites interact more potently with targets than the metabolites of RBV. ETAR is not expected to induce mutations probably due to its lack of pseudo-base pair presence (Chung *et al*., 2008).

2.5 Virus fusion inhibition

The target of interest of another study was G2 envelope glycoprotein, which plays a role of the viral fusion protein. It seems to be similar with other molecules of class II fusion proteins, as suggested *in silico* and *in vitro* analyses (Cifuentes-Muñoz *et al.*, 2011; Tischler *et al.*, 2005). Its ectodomain is composed of three domains which are connected by a stem to the anchor in the viral envelope. It has been shown, that these fusion proteins could be inhibited by protein fragments spanning domain III (DIII) and the stem region. For this reason, recombinant ANDV DIII and stem peptides were synthesized and expected to inhibit membrane fusion and cell entry. Combination of DIII and the C-terminal part of stem region inhibited the infection of Vero E6 by ANDV up to 60% during the endosomal route of ANDV. When fusion of ANDV occurred at the plasma membrane, infection was inhibited over 95%. According to these results, a strategy using hantavirus stem fragments may obviously inhibit fusion of similar viruses within the same genus (Barriga *et al.*, 2016).

2.6 Immunotherapy

Presently, there are no published reports of controlled clinical use of immunotherapy for HFRS and HCPS in humans. Some studies in animal models (hamsters, mice and rats) indicated that passive administration of neutralizing antibodies (Abs) or polyclonal sera to HTNV can sufficiently protect animals from disease involved with the same species of virus (Zhang *et al.*, 1989; Arikawa *et al.*, 1992; Xu *et al.*, 2002). Anti-HTNV G2-specific neutralizing Abs administrated 4 dpi sufficiently protected hamsters and up to 2 dpi protected suckling mice from lethal outcome (Linag *et al.*, 1996; Xu *et al.,* 2002). Post-exposure administration of neutralizing Abs was demonstrated against HCPS-causing hantaviruses, as well. Immune plasma obtained from HCPS patients infected by ANDV and SNV protected hamsters and deer mice infected by homologous virus, respectively (Custer *et al*., 2003; Medina *et al.*, 2007).

3. Host-targeting antivirals

3.1 Corticosteroid therapy

High levels of proinflammatory cytokines, especially TNF-α were detected in sera of patients with HFRS and HCPS. TNF-α is released by neutrophils, NK cells, $CD8^+$ T cells as well as DC and macrophages infected by a hantavirus (Schönrich *et al*., 2015; Kilpatrick *et al*., 2004). An immunomodulatory treatment was firstly performed and evaluated during the Korean war, when oral or intramuscular application of corticoids reduced lethal cases of HFRS due to the shock, but the mortality was not decreased at all (Sayer *et al.*, 1955).

A retrospective analysis of 22 HCPS patients in Chile noted that high-dose methylprednisolone treatment reduced mortality during the shock (Tapia *et al*., 2000). Another study involved 60 Chilean patients with HCPS caused by ANDV. This study reported a phase 2, double-blind, placebo-controlled clinical trial to evaluate the parameters such as the safety and the efficacy of intravenously applied methylprednisolone in patients with HCPS in Chile. The treatment of HCPS with high-dose methylprednisolone seems to be safe, but it is not recommended for clinical use, because there was no significant difference in lethal outcome between the methylprednisolone recipients (8 of 30 patients - 27%) and placebo recipients (12 of 30 patients – 40%) (Vial *et al*., 2013).

3.2 Host-cell hantavirus-binding receptor inhibitors

Pathogenic hantaviruses attach to the surface of host cells using their $\alpha_v\beta_3$ integrins. For this reason, a couple of synthesized cyclic nonapeptides, CLVRNLAWC and CQAT TARNC were designed, and found to inhibit SNV infection *in vitro* at a 4:1 nanoparticle-to-virus ratio (9.0% to 32.5% and 27.6% to 37.6%, respectively). CQATTARNC used at a 20:1 ratio, inhibited infection by 50% (Hall *et al.*, 2008). Another peptidomimetic compounds were chosen on the base of their molecular structure and possible ability to bind $\alpha_v\beta_3$ cell receptor. Forty nine peptidomimetic molecules in the first round and 68 molecules in the second round of screening with antihantavirus effect in the two thousand lower micromolar range were identified. In result, a unique set of chemical compounds for the next phases of the drug discovery development was obtained. Their antiviral potential needs to be refined and supported by *in vivo* studies (Hall *et al.*, 2010).

3.3 Therapy via blocking of bradykinin B2 receptor

Another promising idea for the therapy of hantavirus diseases is the use of bradykinin receptor antagonists. Increased capillary permeability and vascular leakage are typical for all hantavirus infections. Complement activation seems to be linked to vascular changes in PUUV infections. The mechanisms behind the changes of vascular permeability after hantavirus infection are obviously a multifactorial event which is not yet completely described. It has been found that hantaviruses are responsible for increased activation of the kinin-kallikrein system during the infection of endothelial cells, resulting in the liberation of bradykinin (Bossi *et al.*, 2004; Golias *et al.*, 2007; Taylor *et al.*, 2013).

Bradykinin is a nonapeptide binding bradykinin B2 receptor in role of an inflammatory mediator which is responsible for a dilatation of the blood vessels, increased vascular permeability and subsequently causes the blood pressure to fall. Icatibant is a peptidomimetic drug which is a selective antagonist of bradykinin B2 receptors. Icatibant blocks the binding of bradykinin to the bradykinin B2 receptor by binding to this receptor itself (Taylor *et al.*, 2013).

A case report described a 37-year-old Finnish male patient with severe PUUV infection successfully treated with a single dose of icatibant (Antonen *et al.,* 2013; Vaheri *et al.*, 2014). A report of another case, a 67-year-old female patient with severe HFRS caused by PUUV described a patient with a malignant chronic lymphoproliferative disease mostly affecting the spleen. In addition, patients' blood disease was morphologically considered as either atypical chronic lymphotic leukemia (CLL) or splenic marginal zone lymphoma. The 2 day delay between the doses the icatibant had no significant role in the recovery. Although this patient did not die, the icatibant did not play the role in recovery. Icatibant was not sufficient probably due to an extremely severe case of PUUV infection. It can be useful to note that one of the predictions of severity of disease is a spleen with abnormalities. Nevertheless, the bradykinin B2 receptor antagonist icatibant is surely worth a further study as a target in the treatment of severe hantavirus infections (Laine *et al.*, 2015).

4. Vaccines

There are no Food and Drug Administration (FDA)-licensed vaccines for HFRS or HCPS. Some vaccines based on use of immunoactive inactivated virus particles are in use in Far East, particularly in China and Korea. Other approaches of vaccine development were also studied and evaluated. Recently, some excellent review articles about vaccines against hantaviruses have been already published (Maes *et al.*, 2009; Schmaljohn, 2009, 2012; Krüger *et al.*, 2011), so we focused mostly on the most recent highlights.

4.1 Inactivated virus vaccines

Anti-hantavirus inactivated vaccines used in China and Korea are generally inactivated by formalin or β-propiolactone. These vaccines are aimed to protect against the hantaviruses HTNV, SEOV and PUUV, which are causing most of the cases of HFRS (Zhang *et al.*, 2010). Hantavax™, a formalin-inactivated vaccine developed in Korea consists HTNV amplified in mouse brains. It is commonly used since 90's in Korea and China. Hantavax™ showed immunogenicity lasting at least two years with a three-dose schedule. The protective neutralizing antibody response showed to be sufficient just after third boosting dose (Song *et al.*, 2016).

Except of China and Korea, the research of anti-hantavirus vaccines was established also in Russia. An inactivated bivalent PUUV/DOBV vaccine consisting the hantavirus strains PUUV Ufa-97 and DOBV-Aa Lipetzk-06 was developed. The aluminium hydroxide was used as adjuvant. This vaccine showed a significant neutralizing antibody activity against both PUUV and DOBV in immunized BALB/c mice. This bivalent vaccine against PUUV and DOBV passed pre-clinical tests under the Russian control authority institution and seems to be a promising approach in prevention against these species of hantaviruses (Krüger *et al.*, 2011).

Recently, there are no studies about using of live attenuated hantaviruses for humans. Meanwhile, it has been proposed that a genetic reassortant of pathogenic and non-pathogenic virus species could be a feasible vaccine development. A particle from the S and L segments of Prospect Hill virus and the M segment of PUUV was constructed. This virus particle interacted with elements of the innate immune system *in vitro* as Prospect Hill virus, but because of the PUUV origin of the M segment is expected to induce anti-PUUV neutralizing immune response (Handke *et al.*, 2010).

4.2 Chimeric molecular vaccines

Non-replicating adenovirus vectors showed to be good carriers for a development of recombinant vaccines against hantaviruses ANDV and SEOV as representatives of HCPS and HFRS causing hantaviruses. Adenovirus expressing ANDV N, G1 or G2 proteins sufficiently protected the hamsters against lethal outcome of infection with ADNV (Safronetz *et al.*, 2009). Another model of a replication-competent recombinant canine adenovirus type 2 expressing the G1 protein of SEOV (rCAV-2-G1) in BALB/c mice was evaluated. Sera from immunized mice contained antibodies which specifically recognized SEOV and neutralized it *in vitro*. The recombinant virus completely protected the animals against a lethal challenge with the highly virulent strain of SEOV-CC-2 (Yuan *et al.*, 2009).

4.3 Virus-like particles

Virus-like particles (VLPs), such as hepatitis B virus and polyomavirus core particles, are viral proteins carrying foreign epitopes (Ulrich *et al.*, 1998). HTNV-VLPs by co-expressing HTNV N protein and G1 and G2 glycoproteins in Chinese hamster ovary (CHO) cells were generated. Then, intramuscular and subcutaneous administrations of HTNV-VLPs were compared for the ability to induce a specific immune response against HTNV infection in mice. The vaccination with HTNV-VLPs resulted in the induction of higher levels of specific cellular immune response to N protein in contrast with inactivated vaccine (Li *et al.*, 2010). It has been shown that more species of hantaviruses (ANDV and PUUV) are potent to form VLPs just from G1 and G2 glycoproteins which are pleomorphic and expose protrusions. The viral nucleoprotein was not required for particle formation. These characteristics can be used for inducing of specific immune response for different species of hantaviruses (Acuña *et al.*, 2014).

4.4 DNA vaccines

Different types of DNA vaccines against HTNV, SEOV, PUUV, ANDV and SNV using linear DNA, plasmid DNA and alphavirus replicons carrying genes for N protein and/or glycoproteins have been made. Interestingly, their immunogenicity apparently differs in different animal models. The M segment of ANDV was presented as immunogenic in nonhuman primates and rabbits but not in hamsters (Hammerbeck *et al.*, 2009). Three groups of nine volunteers were vaccinated with DNA vaccines for HTNV, PUUV or with a mixture of both vaccines expressing G1 and G2 genes of these viruses within the phase I study. Hantavirus neutralizing antibodies were detected in five of nine and seven of nine persons who received all three vaccinations with the HTNV or PUUV DNA vaccine. In case of combined vaccine group, seven of the nine participants after all three vaccinations developed antibodies against PUUV. The three strongest responders to the PUUV vaccine had a strong neutralizing response to the HTNV, too. Both, HTNV and PUUV DNA vaccines were immunogenic, but when mixed, more individuals responded to the PUUV in contrast to the HTNV DNA vaccine (Hooper *et al.*, 2014). DNA vaccines protecting from hantaviruses causing HCPS were demonstrated on geese which were vaccinated with an ANDV DNA vaccine encoding the virus envelope glycoproteins for a purpose to produce neutralizing antibodies for use in humans because availability of convalescent plasma from survivors is very limited. Geese are supposed to produce IgY and alternatively spliced IgYΔFc, that can be purified at high concentrations from egg yolks. IgY lacks the mammalian Fc that can create antibodies in horses, sheep, and rabbits reactogenic in humans. All geese developed a high-titer neutralizing antibodies after second vaccination. It was shown by a pseudovirion neutralization assay (PsVNA) that high level of these neutralizing antibodies were maintained for over 1 year. Moreover, a booster vaccination resulted in higher levels of neutralizing antibodies (i.e., $PsVNA_{80}$ titers >100,000). The protective efficacy of the sera was proved in hamster model of lethal HCPS. It was shown that IgY/IgYΔFc purified from eggs transferred to hamsters subcutaneously starting 5 days after IM challenge with ANDV (25 LD_{50}) protected 7 of 8 hamsters. As it was shown, DNA vaccine/goose platform is obviously a good candidate of preventing a lethal HCPS when administered post-exposure (Haese *et al.*, 2015).

5. Concluding remarks

Hantaviruses threaten the people throughout the world by serious diseases. Instead of the only available antiviral drug Ribavirin against the hantaviruses, there are some other promising approaches under development which could significantly sustain the antiviral efficacy of Ribavirin and decrease the lethal endings of both, HFRS and HCPS. The combination therapy of RBV and any other antiviral compound could be apparently more effective in therapy of hantavirus infections than the discrete usage of only one of them. The use of nucleoside and pyrazine derivates as well as peptide derivates binding the cellular $\alpha_v\beta_3$ integrins, the receptors for adsorption of pathogenic hantaviruses could be another kind of effective therapy. However, a development of efficient and safe vaccines seems to be the best option to prevent the dangerous hantavirus diseases.

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